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**A multidisciplinary investigation of underlying pathways in ADHD
A study of ADHD genes, endophenotypes and phenotypes**

Pinto, Rebecca

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Author: Rebecca Pinto

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**A multidisciplinary investigation of underlying pathways in ADHD:
A study of ADHD genes, endophenotypes and phenotypes**

Rebecca Pinto

MRC Social, Genetic and Developmental Psychiatry Centre
Institute of Psychiatry
King's College London

Thesis submitted for degree of Doctor of Philosophy
2012

This is dedicated to my mother, Ima Campos

Abstract

This thesis adopts a multidisciplinary approach, combining cognitive-experimental and physiological data, with quantitative and molecular genetic analyses, to investigate pathways from genes to ADHD behaviours.

In chapter 2 we investigated socio-demographic factors as contributors of contrast effects (exaggeration of behavioural differences) in parental ADHD ratings. Gender moderated contrast effects, but only in opposite-sex pairs. Family size also contributed to contrast effects, which was further modified by gender. The reliance on rating scales and contrast effects may undermine gene-finding efforts. Accordingly, interest has been directed at objective ADHD-related measures. Promising candidates include heightened reaction time variability (RTV) and inhibitory deficits, indexed by commission errors (CE). Using a population-based twin sample we identified RTV and CE as separate cognitive pathways underlying inattention and hyperactivity-impulsivity (chapter 3). Molecular genetic investigations in the same sample (chapter 4) identified overlapping associations in line with these findings. However, no associations survived correction for multiple testing or were replicated in analysis of a clinical sample; we therefore cannot discount the possibility that they reflect chance findings.

Behaviours that frequently co-occur with ADHD were investigated to elucidate shared versus unique pathways, and moderators. We found that social autistic-like traits (ALTs) largely underlie the covariation of ADHD behaviours and ALTs (chapter 5), and observed significant phenotypic and genetic covariation between RTV and social ALTs. In chapter 6, we investigated the aetiological covariation of ADHD and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, indexed by salivary cortisol. Using growth curve modelling, we identified an association between ADHD affection status and rate of change (declined faster). Further analysis suggested that this association was primarily driven by oppositional behaviours, and that there was a familial component underlying this covariation.

Overall, the research summarised in this thesis will facilitate the further development of causal models linking genetic variation via mediating processes to ADHD behaviours.

Statement of work

The present thesis uses data collected from four studies. The research described in chapter 2 utilised data from the Twins' Early Development Study (TEDS); an ongoing general population cohort of twins headed by Professor Robert Plomin (funded by the UK Medical Research Council (MRC) grant G0901245 and G0500079 to Professor Robert Plomin). Chapters 3 to 5 were based on data collected from two studies. The first of these studies was the Study of Activity and Impulsivity Levels (SAIL) in children, headed by Dr Jonna Kuntsi (funded by a Wellcome Trust grant GR070345MF to Dr Jonna Kuntsi). The SAIL sample is a subsample recruited from TEDS. The second sample is an ADHD-proband sibling-pair sample (the London part of this project is funded by MRC grant G03001896 to Dr Jonna Kuntsi; the international collaboration with seven other teams from the International Multicentre ADHD Genetics Consortium (IMAGE) was funded, in part, by NIMH grant R01062873 to Professor Steve Faraone (London PI: Professor Philip Asherson)). I was not involved in the planning or collection of data for the above studies.

The research described in chapter 6 is based on a selected sample of male adolescent twin pairs (NEurophysiology of Attention and Activity in Twins (NEATT); PI: Professor Robert Plomin and Dr Grainne McLoughlin), recruited from TEDS for consistently high/low parental ADHD ratings. Participants were invited to the research centre for cognitive-electroencephalography assessment and collection of salivary cortisol. I played a key role in the conception and design of the salivary cortisol component of the project, under the supervision of Dr Jonna Kuntsi and Professor Philip Asherson. I was involved in writing up the ethics application for the salivary cortisol component of the project, collecting salivary cortisol samples, collecting questionnaire data from both twin pairs and parents, and conducting expressed emotion tasks with parents (not included in thesis).

The research questions were developed by myself, both my supervisors Dr Jonna Kuntsi and Dr Frühling Rijdsdijk, and also Professor Philip Asherson. Analysis was conducted by myself under the supervision of Dr Frühling Rijdsdijk and Dr Jonna Kuntsi, Dr Nick Iliott (Chapter 4) and Professor Philip Asherson (Chapter 4). All work presented in this thesis is original and my own. The interpretations of the results are mine, reached through discussions with Dr Jonna Kuntsi, Dr Frühling Rijdsdijk, and Professor Philip Asherson.

Publications

Chapter 2 is adapted from:

Pinto, R., Rijdsdijk, F., Wood, A.C., Asherson, P., & Kuntsi, J. (2012). Bigger families fare better: a novel method to estimate rater contrast effects in parental ratings on ADHD symptoms. *Behavior Genetics*; DOI: 10.1007/s10519-012-9561-y

Chapter 3 is adapted from:

Kuntsi, J.,* **Pinto, R.,*** Price, T.S., van der Meere, J., Wood, A.C., Asherson, P. (under review). The separation of ADHD inattention and hyperactivity-impulsivity symptoms: pathways from genetic effects to cognitive impairments and symptoms. *Psychological Medicine*

* Joint first authors

Chapter 4 is adapted from:

Pinto, R., Rijdsdijk, F., Price, T.S., Illot, N., Asherson, P., Cheung, C., & Kuntsi, J. (in preparation). QTL analysis of putative ADHD risk markers using quantitative behavioural measures of ADHD symptoms and cognitive impairments

Chapter 5 is adapted from:

Pinto, R., Rijdsdijk, F., Asherson, P., & Kuntsi, J. (in preparation). Genetic overlap between ADHD behaviours and autistic-like traits.

Chapter 6 is adapted from:

Pinto, R., Rijdsdijk, F., Ouellette-Morin, I., Asherson, P., McLoughlin, G., & Kuntsi, J. (in preparation). ADHD symptoms and atypical hypothalamic-pituitary-adrenal axis functioning: evidence for moderating effects of oppositional behaviours and a familial component

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Table of contents

ABSTRACT	3
STATEMENT OF WORK	4
PUBLICATIONS	5
ACKNOWLEDGEMENTS.....	6
TABLE OF CONTENTS	7
LIST OF TABLES.....	15
LIST OF FIGURES.....	17
LIST OF ABBREVIATIONS.....	17
CHAPTER 1 THE ADHD PHENOTYPE AT A BEHAVIOURAL, GENETIC, COGNITIVE, AND PHYSIOLOGICAL LEVEL.....	20
1.1 ABSTRACT	20
1.2 ADHD AS A CLINICAL DISORDER	21
1.2.1 Historical context of the development of ADHD.....	21
1.2.1.1 Historical origins of a disorder resembling ADHD	21
1.2.1.2 Diagnostic origins of ADHD	22
1.2.1.3 Current classification	24
1.2.1.4 On-going refinement of operational diagnostic criteria	24
1.2.2 ADHD epidemiology	25
1.2.2.1 Prevalence of ADHD.....	25
1.2.2.2 Gender differences in ADHD.....	26
1.2.2.3 Developmental trajectories of ADHD	27
1.2.2.4 Psychiatric comorbidity.....	29
1.2.3 Summary of ADHD as a clinical disorder	30
1.3 BEHAVIOURAL GENETIC STUDIES OF ADHD	30
1.3.1 Clinical family studies	30
1.3.2 Overview of the twin method	31
1.3.2.1 The classical twin design	33

1.3.2.2 Path diagrams	35
1.3.2.3 Multivariate genetic analysis	38
1.3.2.4 The Cholesky Decomposition.....	39
1.3.2.5 Correlated factors solution of the Cholesky Decomposition	40
1.3.2.6 Limitations of the twin method	42
1.3.3 ADHD as a quantitative trait	43
1.3.4 The genetic and environmental aetiology of ADHD symptoms.....	45
1.3.4.1 The genetic aetiology of ADHD symptoms	45
1.3.4.2 The environmental aetiology of ADHD symptoms	49
1.3.5 Longitudinal studies	47
1.3.6 Aetiological sex differences.....	48
1.3.7 Informant effects.....	49
1.3.7.1 Informant effects: parent versus teachers	49
1.3.7.2 Low DZ correlations: rater contrast effects	49
1.3.7.3 Teacher ratings: same- versus different-teacher ratings.....	51
1.3.7.4 Rater disagreement	53
1.3.8 Assessment instrument effects.....	55
1.3.9 Genetic overlap between inattention and hyperactivity symptom domains	56
1.3.10 Summary of ADHD twin studies	57
1.4 ADHD AETIOLOGY.....	57
1.4.1 Genetic risk factors.....	57
1.4.1.1 Linkage studies.....	58
1.4.1.2 Candidate gene association studies.....	58
1.4.1.3 Quantitative trait loci (QTL) approach	59
1.4.1.4 Genome-wide association studies (GWAS).....	61
1.4.1.5 Summary of molecular genetic progress and future directions	62
1.4.2 Environmental factors	63
1.4.3 GxE interactions	63
1.4.4 Summary of ADHD aetiological risk factors	64
1.5 COGNITIVE THEORETICAL MODELS OF ADHD	64
1.5.1 Executive dysfunction theory of ADHD	64
1.5.2 Arousal dysfunction theories of ADHD.....	66

1.5.2.1 The state-regulatory model	66
1.5.2.2 The arousal-attention model	67
1.5.3 The neurodevelopmental model of ADHD	68
1.5.4 Summary of cognitive theoretical models of ADHD	69
1.6 ENDOPHENOTYPES.....	70
1.6.1 Criteria 1: Endophenotype must be reliable	73
1.6.2 Criteria 2: Endophenotype should demonstrate heritability	73
1.6.3 Criteria 3: Endophenotype should correlate with clinical disorder/behavioural symptoms.....	74
1.6.4 Criteria 4: Endophenotypes should share common genetic influences with clinical disorder/behavioural symptoms.....	76
1.6.4.1 Evidence for shared common genetic influences: quantitative genetic studies.....	76
1.6.4.2 Evidence for shared common genetic influences: molecular genetic studies.....	77
1.6.5 Summary	78
1.7 THE LINK BETWEEN ADHD AND ASD.....	79
1.7.1 ASDs.....	79
1.7.2 Clinical overlap between ADHD and ASD	79
1.7.3 Familial overlap: autistic-like traits in families of ADHD probands.....	80
1.7.4 Genetic overlap between ADHD and ASD	81
1.8 ADHD AND ATYPICAL HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS ACTIVITY.....	82
1.8.1 The HPA axis.....	82
1.8.2 The HPA axis and adverse health outcomes	82
1.8.3 The HPA axis and ADHD	83
1.9 INTERIM SUMMARY	85
1.10 SPECIFIC AIMS AND OBJECTIVES OF THESIS	85
CHAPTER 2 BIGGER FAMILIES FARE BETTER: A NOVEL METHOD TO ESTIMATE RATER CONTRAST EFFECTS IN PARENTAL RATINGS ON ADHD SYMPTOMS.....	88
2.1 ABSTRACT.....	88
2.2 INTRODUCTION	88
2.3 METHODOLOGY	91

2.3.1 Sample	91
2.3.2 Measures	92
2.3.2.1 ADHD symptoms	92
2.3.2.2 Socio-demographic factors	93
2.3.3 Analyses.....	96
2.3.3.1 Step 1: Testing variance differences according to zygosity to indicate possible contrast effects (twin correlation model without sibling interaction parameters)	96
2.3.3.2. Step 2: Testing if contrast effects significantly differ between same-sex and opposite-sex pairs according to gender (twin correlation model with total sibling interaction parameter)	96
2.3.3.2.1 The rater contrast model.....	96
2.3.3.3 Step 3: Testing moderators of contrast effects (twin correlation model with independent and moderator-dependent sibling interaction effects)	99
2.4 RESULTS.....	99
2.4.1 Testing variance differences according to zygosity to indicate possible contrast effects.....	101
2.4.2 Testing if contrast effects significantly differ between same-sex and opposite-sex pairs according to gender	101
2.4.3 Testing moderators of contrast effects.....	104
2.4.3.1 Moderating effects of gender.....	104
2.4.3.2 Interactive effect of gender and other socio-demographic moderators on contrast effects.....	104
2.5 DISCUSSION.....	108
CHAPTER 3 THE SEPARATION OF ADHD INATTENTION AND HYPERACTIVITY-IMPULSIVITY SYMPTOMS: PATHWAYS FROM GENETIC EFFECTS TO COGNITIVE IMPAIRMENTS AND ADHD SYMPTOMS.....	112
3.1 ABSTRACT	112
3.2 INTRODUCTION	113
3.3 METHODOLOGY	115
3.3.1 Sample and procedure	115
3.3.2 Measures.....	116

3.3.2.1 Behavioural rating scales	116
3.3.2.2 Wechsler Intelligence Scales for Children.....	117
3.3.2.3 The go-no/go task	117
3.3.2.4 The fast task.....	117
3.3.2.5 Selection of cognitive variables for analyses	118
3.3.3 Analyses.....	118
3.3.3.1 Structural equation modelling.....	118
3.3.3.2 Overview of the twin method.....	120
3.3.3.3 Univariate genetic analyses	120
3.3.3.4 Parameter selection for multivariate models informed by univariate genetic analyses.....	122
3.3.3.5 Multivariate genetic analyses	122
3.4 RESULTS.....	124
3.4.1 Phenotypic and aetiological association between cognitive impairments and the two symptom domains of ADHD considered separately.....	127
3.4.2 Aetiological association between RTV and inattention, independent of hyperactivity-impulsivity	131
3.5 DISCUSSION.....	133
CHAPTER 4 QTL ANALYSIS OF PUTATIVE ADHD RISK MARKERS USING QUANTITATIVE BEHAVIOURAL MEASURES OF ADHD SYMPTOMS AND COGNITIVE IMPAIRMENTS	136
4.1 ABSTRACT.....	136
4.2 INTRODUCTION	137
PART A: A GENERAL POPULATION TWIN SAMPLE	140
4.3 METHODOLOGY	141
4.3.1 Sample and procedure	141
4.3.2 Measures.....	141
4.3.3 Selection of variables to take forward for molecular genetic analyses	141
4.3.4 Genotyping.....	142
4.3.5 Analyses.....	143
4.4 RESULTS.....	146
4.4.1 Association analyses.....	146

4.4.2 Testing candidate mediating pathways	149
PART B: AN ADHD-PROBAND AND CONTROL SIBLING-PAIR SAMPLE.....	150
4.5 METHODOLOGY	150
4.5.1 Sample and procedure	150
4.5.2 Measures.....	151
4.5.3 Genotyping and analyses	152
4.6 RESULTS.....	152
4.7 DISCUSSION.....	158
CHAPTER 5 GENETIC OVERLAP BETWEEN ADHD BEHAVIOURS AND AUTISTIC-LIKE TRAITS	163
5.1 ABSTRACT	163
5.2 INTRODUCTION	164
5.3 METHODOLOGY	167
5.3.1 Sample and procedure	167
5.3.2 Measures.....	168
5.3.2.1 Behavioural rating scales	168
5.3.2.2 Wechsler Intelligence Scales for Children.....	168
5.3.2.3 The go-no/go task	168
5.3.2.4 The fast task.....	168
5.3.2.5 Selection of variables for analyses.....	168
5.3.3 Analyses.....	170
5.3.3.1 Structural equation modelling	170
5.3.3.2 Univariate genetic analyses	170
5.3.3.3 Multivariate genetic analyses	170
5.4 RESULTS.....	171
5.4.1 The phenotypic and genetic associations with social and non-social ALTs and the two ADHD symptom domains of inattention and hyperactivity-impulsivity.....	175
5.4.2 The phenotypic and genetic associations with social and non-social ALTs and RTV and CE	175
5.4.3 Aetiological association between social ALTs and inattention, independent of RTV	177
5.5 DISCUSSION.....	179

**CHAPTER 6 ADHD AND ATYPICAL HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS
FUNCTIONING: EVIDENCE FOR FAMILIAL OVERLAP AND MODERATING EFFECTS
OF OPPOSITIONAL BEHAVIOURS.....182**

6.1 ABSTRACT	182
6.2 INTRODUCTION	183
6.3 METHODOLOGY	186
6.3.1 Sample and procedure	186
6.3.2 Measures	186
6.3.2.1 Behavioural rating scales	186
6.3.2.2 Salivary cortisol	187
6.3.3 Analyses.....	189
6.3.3.1 Group mean differences	189
6.3.3.2 Genetic model fitting analysis.....	189
6.3.3.2.1 Relationship of ADHD affection status and indices of cortisol activity.....	189
6.3.3.2.2 Genetic Growth Curve Models (GCM)	190
6.3.3.2.3 Multivariate genetic model of ADHD affection status, oppositional behaviours and derived slope factor scores	192
6.4 RESULTS.....	193
6.4.1 Group mean differences	193
6.4.2 Genetic model fitting analyses.....	198
6.4.2.1 Relationship between ADHD affection status and indices of cortisol activity.....	198
6.4.2.2 Testing main effects of ADHD affection status on intercept and slope factors	201
6.4.2.3 Testing main effects of ADHD affection status on growth curve factors while controlling for covariates and modelling moderating effects of anxiety-shy and oppositional behaviours	201
6.4.2.3.1 Aetiological components of growth curve factors.....	202
6.4.2.3.2 Aetiological components of the individual cortisol measures.....	202
6.4.2.4 Multivariate genetic model of ADHD affection status, oppositional behaviours and slope factor scores	204

6.4.2.5 Aetiological association between oppositional behaviours and the slope factor scores, independent of ADHD affection status	207
6.5 DISCUSSION.....	209
CHAPTER 7 GENERAL DISCUSSION	213
7.1 ABSTRACT.....	213
7.2 SUMMARY OF MAJOR FINDINGS.....	215
7.3 WIDER IMPLICATIONS FOR ADHD RESEARCH	215
7.3.1 Increased knowledge of factors contributing to the inaccuracy of parental ADHD ratings	215
7.3.2 Validation of the dimensional approach to ADHD	216
7.3.3 Supporting the separation of ADHD symptom subscales	217
7.3.4 Contribution to cognitive theoretical models of ADHD	218
7.3.5 Clarifying the link between ADHD and ASD	218
7.3.6 RTV and CE as ADHD endophenotypes	219
7.3.7 The identification of potential new endophenotypes	219
7.4 STRENGTHS AND LIMITATIONS	220
7.4.1 Limitations of the twin method.....	220
7.4.2 Sample characteristics and measurement issues	221
7.5 FUTURE DIRECTIONS	222
7.6 OVERALL CONCLUSION.....	223
REFERENCES.....	226
APPENDIX A: TABLE A.1 GENETIC MARKERS CHOSEN FOR GENOTYPING IN POPULATION-BASED TWIN SAMPLE	252
APPENDIX B: TABLE B.1 MAXIMUM-LIKELIHOOD CORRELATIONS	253
APPENDIX B: TABLE B.2 AETIOLOGICAL AND PHENOTYPIC CORRELATIONS.....	254
APPENDIX C: SUPPLEMENTARY INFORMATION ON GROWTH MIXTURE MODELLING ON LONGITUDINAL TEDS ADHD DATA	256

List of tables

Table 1.1 Current (DSM-IV-TR) symptom checklist for ADHD	223
Table 1.2 Significant ($p < 0.05$) meta-analytic results for associations between candidate gene polymorphisms and childhood ADHD.....	60
Table 2.1 Number of twin pairs by zygosity and gender, by socio-demographic variables	95
Table 2.2 Means (and variances) by sex-zygosity groups	100
Table 2.3 Twin correlations and variance estimates by zygosity: testing zygosity differences in variances	102
Table 2.4 Contrast effect parameters between same-sex and opposite-sex pairs, by gender	10103
Table 2.5 Contrast effect parameters (and 99% confidence intervals) by gender composition of twin pairs, decomposed into independent and moderator-dependent components .	105
Table 3.1 Means and standard deviations.....	125
Table 3.2 Twin pair correlations (and 95% confidence intervals).....	126
Table 3.3 Phenotypic correlations and standardised parameter estimates (with 95% confidence intervals) from the correlated factors solution of the full Cholesky Decomposition, within and across ADHD behavioural ratings and cognitive measures	129
Table 4.1 QTDT association analysis in a population-based twin sample	148
Table 4.2 QTDT association analysis of SNPs spanning SLC6A2 in clinical proband and sibling sample.....	153
Table 4.3 QTDT association analysis of SNPs spanning 5HT2A in clinical proband and sibling sample.....	156
Table 5.1 Items used to measure autistic-like traits by social and non-social symptom subscales	169
Table 5.2 Means and standard deviations for behavioural ratings and cognitive measures	172
Table 5.3 Maximum-likelihood CTCT correlations (constrained correlated model), and aetiological and phenotypic correlations (standardised correlated factors solution	

genetic ACE model) for social and non-social ALTs between ADHD behaviours and cognitive measures	17173
Table 6.1 Characteristics of the sample	196
Table 6.2 Cross-twin within-trait correlations for cortisol composite measures; Cross-twin cross-trait correlations for ADHD affection status and cortisol composite measures; Phenotypic correlations between ADHD affection status and cortisol composite measures.....	199
Table 6.3 Aetiological components for individual cortisol sampled during cognitive-EEG session	204

List of figures

Figure 1.1 The three classical univariate twin models.....	37
Figure 1.2 Bivariate Cholesky ADE Decomposition.....	39
Figure 1.3 Bivariate correlated factors solution of the ADE Cholesky Decomposition.....	41
Figure 2.1 The variance-covariance model of MZ/DZ twin pairs by varying gender composition	98
Figure 3.1 Correlated factors solution of the full Cholesky Decomposition.....	128
Figure 3.2 Additive genetic and individual-specific environmental parameter estimates from the three-variable Cholesky Decomposition.....	132
Figure 4.1 Path diagram for a mediation model.....	145
Figure 4.2 Model depicting the pleiotropic effects of SLC6A2 (upper model) and the direct effects of 5HT2A on inattention and the indirect effect via RTV (lower model) in population-based twin sample	161
Figure 5.1 Correlated factors solution of the full ACE Cholesky Decomposition.....	176
Figure 5.2 Broad-sense genetic and individual-specific environmental parameter estimates from the three-variable Cholesky Decomposition	178
Figure 6.1 Mean salivary cortisol concentration by group status during cognitive testing (upper panel) and measuring diurnal variation (lower panel)	195
Figure 6.2 Genetic GCM for cortisol samples collected during cognitive-EEG testing and moderating effects of sampling conditions (age) and oppositional behavioural ratings incorporated as moderators in Intercept and Slope factor means	20203
Figure 6.3 Correlated factors solution of the full Cholesky Decomposition across ADHD affection status, oppositional behaviours and slope factor scores	205
Figure 6.4 Cholesky Decomposition of ADHD affection status, oppositional behaviours and slope growth curve factor scores.....	206

List of abbreviations

ADHD	Attention deficit/hyperactivity disorder
ADHD-C	ADHD Combined subtype
ADHD-PHI	ADHD Predominantly Hyperactivity/Impulsivity subtype
ADHD-PI	ADHD Predominantly Inattentive subtype
ADRA2A	Adrenergic receptor α -2A (ADRA2A) gene
ALTs	Autistic-like traits
ASD	Autistic spectrum disorders
AUC	Area under the curve
CAR	Cortisol awakening response
CBCL	Child Behavior Checklist
CD	Conduct disorder
CDH13	Cadherin 13 gene
CE	Commission errors
CNV	Copy number variants
COMT	Catechol-O-methyltransferase gene
CPRS-R	Revised Conners' Parent Rating Scale
CPT	Continuous Performance Test
CSBQ	Children's Social Behavior Questionnaire
DAT1	Dopamine transporter gene
DRD4	Dopamine D4 receptor gene
DRD5	Dopamine D5 receptor gene
DSM	Diagnostic and Statistical Manual
DZ	Dizygotic
DZOS	Dizygotic Opposite Sex
DZSS	Dizygotic Same Sex
EF	Executive Functioning
GCM	Growth Curve Model
GNG	Go/No-Go task
GWAS	Genome-wise association studies
HFA	High functioning autism
HKD	Hyperkinetic disorder

HPA	Hypothalamic-pituitary-adrenal
ICD	International Classification of Disorders
IMAGE	International Multi-Centre ADHD Genetics Study
MRT	Mean reaction time
MZ	Monozygotic
NEATT	NEurophysiology of Attention and Activity in Twins Study
ODD	Oppositional defiant disorder
OE	Omission errors
OPP	Oppositional behaviours
PDD	Pervasive developmental disorder
PDD-NOS	Pervasive developmental disorder- not otherwise specified
QTL	Quantitative trait loci
RRPSPS	Revised Parent Rutter Scale for Pre-School Children
RT	Reaction time
RTV	Reaction time variability
SAIL	Study of Activity and Impulsivity Levels in children
SCQ	Social Communication Questionnaire
SDQ	Strengths and Difficulties Questionnaire
SES	Socio-economic status
SLC6A2	Norepinephrine transporter gene
SNAP-25	Synaptosomal-associated protein 25 gene
SNP	Single nucleotide polymorphism
TEDS	Twins' Early Development Study
TPH2	Tryptophan hydroxylase 2 gene
VNTR	Variable Number Tandem Repeat
5HT1B; HTR1B	Serotonin 1B receptor gene
5HTT; SLC6A4	Serotonin transporter gene
5HT2A	Serotonin receptor gene

CHAPTER 1 THE ADHD PHENOTYPE AT A BEHAVIOURAL, GENETIC, COGNITIVE, AND PHYSIOLOGICAL LEVEL

1.1 Abstract

Attention-deficit/hyperactivity disorder (ADHD) is one of the most prevalent childhood-onset neurodevelopmental disorders (Stergiakouli & Thapar, 2010), characterised by developmentally inappropriate and impairing levels of inattention and hyperactivity-impulsivity. ADHD aetiology is complex, likely influenced by the additive and interactive effects of both genetic (nature) and environmental (nurture) factors (Martin, McDougall, & Hay, 2008). Twin studies have established a strong genetic component, and the majority of progress to date in identifying common ADHD genetic susceptibility loci has come from candidate gene association studies. However, much of the genetic variance of ADHD remains unaccounted for (Kuntsi, Neale, Chen, Faraone, & Asherson, 2006; Plomp, Van Engeland, & Durston, 2009; Purper-Ouakil, Ramoz, Lepagnol-Bestel, Gorwood, & Simonneau, 2011), as promising genetic variants that retain significance in meta-analyses confer a significant, but small, increased risk (e.g. Gizer, Ficks, & Waldman, 2009). The current generation of molecular genetic studies are increasingly focussing on unravelling the mechanisms by which genetic risk variants contribute to clinical phenotypes, by elucidating gene functionality (Plomin, DeFries, McClearn, & McGuffin, 2008). Furthermore, the investigation of objectively measured intermediate phenotypes may contribute to delineating underlying pathophysiological processes. Elucidating ADHD aetiology and mediating processes are likely to facilitate ADHD prediction, classification, diagnosis, intervention, prevention, and management (Plomin et al., 2008).

The overarching aim of this thesis is to identify pathways from genes to ADHD-related behaviours, adopting a multidisciplinary approach combining both quantitative genetic and molecular genetic analytical approaches to behavioural, cognitive-experimental, physiological, and genotyping data. A second aim is to investigate whether identified neuropsychological and physiological processes are unique to ADHD, or shared with and/or moderated by other commonly co-occurring behaviours.

The first chapter of this thesis provides a selective introduction to ADHD. Following an overview

of ADHD epidemiology, the aetiology of ADHD behaviours (particularly the genetic component) is considered, with a selective overview of ADHD twin studies (and an introduction to the twin method and multivariate genetic analyses included in this thesis) and molecular genetic studies. The subsequent section outlines cognitive-related theoretical models of ADHD. Following this, cognitive impairments included in this thesis are evaluated according to agreed criteria for candidate endophenotypes. Discussion will then shift towards an overview of the clinical and familial overlap of ADHD and autism spectrum disorders (ASDs). I then discuss a newly emerging area of research in ADHD physiology, providing a selective overview of studies investigating hypothalamic-pituitary-adrenal (HPA) axis functioning in ADHD. This chapter will then conclude with the underlying rationale of this thesis, outlining aims and hypotheses.

1.2 ADHD as a clinical disorder

1.2.1 Historical context of the development of ADHD

1.2.1.1 Historical origins of a disorder resembling ADHD

A book on mental disorders published in 1798 by the Scottish physician Sir Alexander Crichton (1763-1856), contains one of the earliest documentations of attention-deficit/hyperactivity disorder (ADHD) related behaviours, specifically impaired attention, termed ‘Mental Restlessness’ (Lange, Reichl, Lange, Tucha, & Tucha, 2010). However, the historical origins of modern day scientific inquiry of a disorder resembling current ADHD is conventionally attributed to a series of lectures delivered to the Royal College of Physicians by George Still (1868-1941) in 1902 (Barkley, 1990; Taylor, 2011). In these lectures a group of children presenting with deficits in inhibitory volition and moral control were described by Still, exhibiting “abnormal incapacity for sustained attention, restlessness, fidgetiness” (as cited in Stefanatos & Baron, 2007, p.6). These behaviours were considered as potentially originating from brain dysfunction (Lange et al., 2010).

The association with impaired brain functioning gained credence in the early 20th century, as children that survived the encephalitis pandemics of 1917-1918 were noted to experience subsequent problem behaviours, such as hyperactivity (Rowland, Lesesne, & Abramowitz, 2002). The concept of an organic aetiology underlying behavioural disorders persisted, although it was increasingly recognised that behavioural disturbances could arise even when

there was no evidence of an organic source, reflected by shifting terminology from ‘minimal brain damage’ to ‘minimal brain dysfunction’ (Barkley, 1990). After the 1960s, focus shifted to describing the behavioural-based symptomatology of disorders and using labels to reflect this characterisation rather than aetiological mechanisms (Baeyens, Roeyers, & Walle, 2006).

1.2.1.2 Diagnostic origins of ADHD

In 1968, a disorder resembling current ADHD first appeared in official diagnostic nomenclature, with the publication of the second edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-II). The disorder ‘hyperkinetic reaction of children’ reflected a disorder of “overactivity, restlessness, distractibility, and short attention span, especially in young children; the behaviour usually diminishes by adolescence” (American Psychiatric Organisation, 1968, p. 50).

No other psychiatric disorder has undergone the same amount of revision as ADHD (Ostrander, Herman, Sikorski, Mascendaro, & Lambert, 2008). Subsequent developments in the taxonomy of the disorder were mainly characterised by shifts in the predominance given to hyperactivity versus inattention, and corresponding subtype classifications. In the third edition of the DSM (DSM-III) primacy was transferred to inattention, and terminology accordingly changed to ‘attention deficit disorder’ (ADD), with or without hyperactivity (American Psychiatric Organisation, 1980). This heralded a major departure from the conceptualisation in the International Classification of Disease (ICD), which maintained an emphasis on severe hyperactivity as a cardinal feature (Barkley, 1990). Moreover, DSM-III ADD differed from its predecessor as it included a list of symptoms, numerical cut-off scores, and guidelines for age of onset, symptom duration, and exclusionary criteria (Barkley, 1990). In the following revision (DSM-III-R), sub-typing was discarded in favour of a more unitary construct, ADHD, captured by a single list of symptoms and one cut-off score (American Psychiatric Organisation, 1987). In DSM-IV the label ADHD was retained, but symptoms were separated into two behavioural dimensions (inattention and hyperactivity-impulsivity), and additional criteria included cross-situational presence and functional impairments (American Psychiatric Organisation, 1994). The list of symptoms (see Table 1.1) and criteria (see section 1.2.1.3) were retained in the following text revision (DSM-IV-TR) (American Psychiatric Organisation, 2000).

Table 1.1 Current (DSM-IV-TR) symptom checklist for ADHD
Inattention:
<ol style="list-style-type: none"> 1. often fails to give close attention to details or makes careless mistakes in schoolwork, work, or other activities 2. often has difficulty sustaining attention in tasks or play activities 3. often does not seem to listen when spoken to directly 4. often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behaviour or failure to understand instructions) 5. often has difficulty organising tasks and activities 6. often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework) 7. often loses things necessary for tasks or activities (e.g., toys, school assignments, pencils, books, or tools) 8. is often easily distracted by extraneous stimuli 9. is often forgetful in daily activities
Hyperactivity-impulsivity:
<p>Hyperactivity</p> <ol style="list-style-type: none"> 1. often fidgets with hands or feet or squirms in seat 2. often leaves seat in classroom or in other situations in which remaining seated is expected 3. often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, it may be limited to subjective feelings of restlessness) 4. often has difficulty playing or engaging in leisure activities quietly 5. often "on the go" or often acts as if "driven by a motor" 6. often talks excessively <p>Impulsivity</p> <ol style="list-style-type: none"> 7. often blurts out answers before questions have been completed 8. often has difficulty awaiting turn 9. often interrupts or intrudes on others (e.g., butts into conversations or games)

1.2.1.3 Current classification

Based on the prevalence of inattentive and/or hyperactivity-impulsivity symptoms, three ADHD subtypes are currently specified: predominantly inattentive (ADHD-PI) subtype (at least six inattentive symptoms are present); predominantly hyperactive-impulsive (ADHD-PHI) subtype (at least six hyperactivity-impulsivity symptoms are present); and combined ADHD (ADHD-C) subtype (at least six inattentive and six hyperactivity-impulsivity symptoms are present). Additional components of current ADHD diagnostic criteria include significant functional impairment across at least two settings (e.g., at home and at school), present before the age of seven and for at least six months. Furthermore, exclusionary criteria states that ADHD cannot be diagnosed alongside a psychotic disorder or a pervasive developmental disorder (PDD), and symptoms should not be better accounted for by another mental disorder.

The current ICD-10 (World Health Organisation, 1992) contains a similar list of symptoms as DSM-IV-TR but does not follow a sub-typing approach, requiring endorsement of all three types of symptoms (inattention, hyperactivity, and impulsivity) for a diagnosis of hyperkinetic disorder (HKD). Consequently, the ICD approach is more restrictive than the DSM, and HKD corresponds most closely to the DSM-IV ADHD-C subtype. Mirroring the DSM, it prohibits a dual diagnosis with PDD, but further restricts a diagnosis alongside conduct disorder (CD), with the alternative 'hyperkinetic conduct disorder.'

1.2.1.4 On-going refinement of operational diagnostic criteria

Revisions are currently ongoing for both DSM (expected to be published in May 2013) and ICD (expected 2015) criteria (Hebebrand & Buitelaar, 2011). Nearly 20 years have elapsed since previous revisions, and accordingly there has been much debate on how our increased understanding of ADHD can be translated in nosology. Despite considerable gains and advances in the genetics and neuroscience of ADHD, the time is still not ripe for incorporating biomarkers to the diagnostic assessment of ADHD (Hebebrand & Buitelaar, 2011). However, of all childhood-onset neurodevelopmental disorders, ADHD is likely to experience the largest revisions in the upcoming DSM (Hebebrand & Buitelaar, 2011).

Proposed revisions to DSM ADHD criteria include increasing the age of symptom onset to age 12, more developmentally appropriate symptom items for adults (see section 1.2.2.3), the inclusion of additional impulsivity items, and dropping the exclusionary criteria of a dual diagnosis of ADHD alongside an autism spectrum disorder (ASD). Further proposed changes include a new 'restrictive inattentive' subtype (at least six inattentive symptoms and no more than two hyperactivity-impulsivity symptoms) (Coghill & Seth, 2011), to capture children with predominantly inattentive symptom presentation but limited hyperactivity/impulsivity symptoms that are currently inadequately captured by diagnostic classification (Nigg, Tannock, & Rohde, 2010). The current diagnostic criteria includes a largely arbitrary symptom cut-off for a diagnostic threshold, therefore there is also an intention to include a dimensional approach to more appropriately capture symptom severity. For the most part, this thesis uses ADHD symptom counts rather than the application of a cut-off for group membership, so that ADHD behaviours are viewed along a severity continuum.

1.2.2 ADHD epidemiology

1.2.2.1 Prevalence of ADHD

Historically there has been a greater utility of ICD-10 HKD criteria in Europe, and DSM-IV ADHD criteria in the United States (US) (Buitelaar et al., 2006). DSM-IV ADHD has a comparatively higher prevalence (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007), potentially contributing to the misconception that ADHD is a predominantly American disorder (Biederman & Faraone, 2005; Faraone, Sergeant, Gillberg, & Biederman, 2003; Moffitt & Melchior, 2007).

A meta-analysis of over 100 studies with a pooled sample of over 170,000 individuals aged 18 or under, revealed no significant prevalence difference between US-based and European studies (Polanczyk et al., 2007), suggesting that the lower ICD-10 HKD prevalence is attributable to its more stringent criteria rather than geographical variation. In fact, the largest source of variation in prevalence rates was attributed to methodological factors (Polanczyk et al., 2007). In addition, prevalence varied according to population variables, such as gender (see section 1.2.2.2) and age (see section 1.2.2.3) (Polanczyk et al., 2007).

Despite some geographical variations, the meta-analysis confirmed ADHD as a worldwide trans-cultural disorder, with an estimated worldwide pooled prevalence of 5.29% (Polanczyk et al., 2007). Standardised future epidemiological studies will allow more accurate estimates to be gleaned, by minimising methodological differences which contribute to prevalence variation (Polanczyk et al., 2007). It is important to note that approximately less than half of the pooled studies in the aforementioned meta-analysis included diagnostic impairment criterion. Accordingly the estimates yielded likely reflect an overestimation of ADHD prevalence, as more stringent diagnostic criteria were not adhered to.

1.2.2.2 Gender differences in ADHD

In the meta-analysis outlined above, prevalence was higher in males compared to females (approximately 10% and 4%, respectively) (Polanczyk et al., 2007). The preponderance of ADHD in males versus females is moderated according to sample ascertainment, with more balanced male-to-female ratios in community-based (1:1 to 3:1) versus clinical (up to 9:1) samples (Skounti, Philalithis, & Galanakis, 2007). The gender discrepancy observed in clinical samples may stem from referral bias (Nussbaum, 2012), potentially due to the less disruptive nature of symptom presentation in females, who are more prone to inattentive symptoms. The relatively equal gender ratio observed in adult ADHD (Nussbaum, 2012) likely stem from girls with ADHD having a higher risk for ADHD-PI subtype, in tandem with the different developmental trajectories of ADHD symptoms (see section 1.2.2.3).

As already alluded to, the gender bias differs according to ADHD subtype. In a nationally representative population-based US sample of over 3000 children, the most pronounced gender bias was found in ADHD-C subtype (Froehlich et al., 2007). A review similarly reported ADHD-C subtype as displaying the most marked male-to-female ratio, in both clinical and community-based samples (Milich, Balentine, & Lynam, 2001). In line with these findings, a recent large-scale epidemiological study in Norway found the most striking gender bias in ADHD-C subtype (Ullebo, Posserud, Heiervang, Obel, & Gillberg, 2012). Furthermore, the gender bias observed across all subtypes, but particularly for ADHD-C subtype, was most marked when based on teacher, as opposed to parent, ratings (Ullebo et al., 2012), suggesting that case identification (at least in teachers) is moderated by gender.

A meta-analysis documented that girls with ADHD tend to have lower rates of comorbid CD and internalising behaviours (e.g., anxiety and depression), compared to their male counterparts (Gaub & Carlson, 1997). ADHD boys from community-based samples displayed comparatively higher internalising and peer aggressive behaviours than girls with ADHD, while similar behavioural rates were found across genders in clinic referred samples, suggesting that gender differences in co-occurring symptoms were further moderated by referral source (Gaub & Carlson, 1997). This implies that girls from clinical samples are more severely impaired, and potentially not representative of the general female ADHD population (Hinshaw, 2002). A subsequent meta-analysis reported conflicting findings: higher internalising behaviours in females with ADHD and no moderation by sample source (Gershon, 2002). It is important to note that these two meta-analyses are old; however, more up-to-date meta-analyses of gender differences in ADHD are currently lacking.

More recently, females with ADHD had higher symptom scores for Child Behavior Checklist (CBCL) withdrawn, anxious depressed, and somatic complaint subscales, compared to their male counterparts (Graetz, Sawyer, Baghurst, & Ettridge, 2006). Moreover, there was an interactive effect between gender and clinical service use, with female non-clinical attendees having lower rates of depressive disorders than female clinical attendees (Graetz et al., 2006). Another study found anxiety-related disorders in females differed according to ADHD subtype, with higher rates of separation anxiety disorders in females with ADHD-PI, and higher rates of generalised anxiety disorder in females with ADHD-C subtype (Levy, Hay, Bennett, & McStephen, 2005).

Although gender differences in ADHD tend to pertain to subtype designation and comorbidity, reviews generally conclude that overall there are more similarities than differences in the behavioural manifestations of ADHD across genders (Rucklidge, 2008; Sassi, 2010).

1.2.2.3 Developmental trajectories of ADHD

Despite originating in childhood, ADHD in adulthood is being increasingly recognised. A cross-national study across 10 countries reported an average of 50% of childhood ADHD cases meet DSM-IV criteria for ADHD as adults (Lara et al., 2009). Retrospectively reported childhood risk

factors identified as independent risk factors associated with persistent ADHD included childhood ADHD-C subtype, comorbid major depressive disorder, the presence of at least three additional childhood co-occurring disorders, parental antisocial personality disorder and paternal anxiety or mood disorder (which includes major depressive disorder and generalized anxiety disorder) (Lara et al., 2009).

A meta-analysis of follow-up studies confirms the persistence of ADHD into adulthood, with 15% of childhood cases meeting full criteria at age 25 (Faraone, Biederman, & Mick, 2006). An additional 50% display a developmental decline in symptoms, but still present with symptomatology associated with significant clinical impairments, meeting criteria of partial remission (Faraone et al., 2006). Follow-up studies suggest the rate of decay is similar for both symptom dimensions during adulthood (Biederman, Petty, Evans, Small, & Faraone, 2010), but during adolescence the symptomatic change is relatively modest for inattentive symptoms, compared to the more pronounced and earlier subsiding rate for hyperactivity-impulsivity symptoms (Mick, Faraone, Biederman, & Spencer, 2004). Further differences include greater temporal instability for ADHD-PHI subtype (Lahey, Pelham, Loney, Lee, & Willcutt, 2005) and a later age of onset for ADHD-PI (Willoughby, Curran, Costello, & Angold, 2000).

A meta-analysis of adult DSM-IV ADHD yielded a pooled prevalence of 2.5% (Simon, Czobor, Balint, Meszaros, & Bitter, 2009). However, the authors noted that this estimate may be conservative, citing that some DSM items are less pertinent to adults and accordingly current criteria is developmental insensitive. Moreover, compared to the demands in childhood within structured settings such as school, adults with ADHD may avoid similarly constrained environments, contributing to less obvious symptom presentation. Further research is needed to clarify the manifestation of ADHD in adulthood and for this to be reflected in diagnostic criteria. As previously outlined, a substantial proportion of individuals demonstrate significant functional impairments in adulthood despite not meeting full diagnostic criteria (Faraone et al., 2006). Accordingly, there have been proposals to revise the diagnostic cut-off point for adult ADHD (to a minimum of four symptoms), in order to efficiently capture all adults presenting with sufficient functional impairment and requiring treatment (Frick & Nigg, 2012).

Overall the evidence suggests the symptomatic persistence of ADHD, and that only a minority of childhood cases of ADHD experience complete remission.

1.2.2.4 Psychiatric comorbidity

ADHD does not tend to present in isolation, with co-occurring disorders the rule rather than the exception (Asherson, 2005). High rates of co-occurring symptoms are reported in population-based samples, despite reflecting a less biased prevalence of comorbidity compared to clinical samples (Elia, Ambrosini, & Berrettini, 2008). A meta-analysis of general population studies estimated odd ratios of 10.7, 5.5, and 3.0, respectively for comorbid CD, depression, and anxiety (Angold, Costello, & Erkanli, 1999). In a recent US-based study of over 60,000 children aged six to 17, amongst those with a parent-reported ADHD diagnosis ($n = 5028$), the majority (67%) had at least one additional parent-reported disorder (compared to 11% of children without ADHD), and 18% had at least three co-occurring disorders (Larson, Russ, Kahn, & Halfon, 2011). Learning disabilities (46%), CD (27%), and anxiety (18%), were the most prevalent co-occurring disorders in children with ADHD (Larson et al., 2011). Furthermore, compared to children with other disorders, ADHD children were the highest users of health and educational services, with service use increasing in a dose-related manner with comorbidity (Larson et al., 2011).

Oppositional defiant disorder (ODD) was reported as the only comorbid disorder that varied by subtype: more prevalent in ADHD-C (51%) and ADHD-PHI (42%), versus ADHD-PI (21%), subtype (Elia et al., 2008). In a sample of pre-adolescent girls with ADHD, no subgroup differences were found for anxiety or mood disorders, although higher rates of ODD and CD were observed in ADHD-C versus ADHD-PI subtype (Hinshaw, 2002).

Persistent ADHD is associated with increased psychiatric comorbidity (Biederman et al., 2010), with one study reporting that 80% of adults with ADHD have co-occurring disorders (Fischer et al., 2007). There is increasing recognition that comorbidity patterns tend to shift developmentally (Thome & Reddy, 2009). In addition, sex differences in adult ADHD are typically related to comorbidity patterns (Fedele, Lefler, Hartung, & Canu, 2012). Female adults with ADHD tend to display higher rates of depressive- and eating-disorders (Rasmussen & Levander, 2009; Sobanski et al., 2007), while male adults with ADHD typically have higher rates of substance-related disorders (Rasmussen & Levander, 2009; Sobanski et al., 2007).

Taken altogether the evidence suggests that ‘pure’ ADHD (no comorbidity) is a rarity, and co-occurring disorders the norm. Moreover, comorbidity patterns tend to differ by gender, age and ADHD subtype.

1.2.3 Summary of ADHD as a clinical disorder

ADHD is one of the most prevalent childhood-onset neurodevelopmental disorders (Stergiakouli & Thapar, 2010). For a majority of childhood cases, ADHD symptoms persist into adulthood, contributing to functional impairments across occupational and social domains (Faraone et al., 2006). Across the lifespan, ADHD is associated with multiple adverse outcomes (Barkley, Fischer, Smallish, & Fletcher, 2006), including a range of co-occurring disorders (Biederman, 2005), contributing to increased service use (Larson et al., 2011).

Taking these factors into account, it is unsurprising that ADHD is associated with large health care costs, with estimates of expenditure in England and Wales of £23 million on initial assessment, and an additional £14 million per year for follow-up care (excluding medication) (King et al., 2006). The high personal, familial and social burden associated with ADHD has accordingly fuelled research, and ADHD ranks amongst one of the most frequently investigated psychopathologies (Nigg, 2006), with particular emphasis directed at uncovering ADHD aetiology.

1.3 Behavioural genetic studies of ADHD

1.3.1 Family studies of clinical ADHD

There is strong evidence to support the assertion that ADHD is highly familial, regardless of diagnostic criteria (see Willcutt 2010 for a review). The increased risk of developing the disorder in first degree relatives of DSM-IV ADHD probands is between six- to eight-fold higher than the general population risk (Willcutt, 2010). However, while family studies have demonstrated the familial aggregation of ADHD, they are unable to distinguish whether this is due to shared genetic and/or environmental factors. Twin studies are ideal to disentangle these aetiological factors, based on the difference in the genetic relatedness of twins: identical (monozygotic, MZ) twins, are genetically identical, and fraternal (dizygotic, DZ) twins, on

average share 50% of their segregating genes. Greater MZ similarity, relative to DZ twins, must be attributed to their greater genetic resemblance (see section 1.3.2 for more details on the classical twin design). Twin studies of clinical ADHD, compare concordance rates according to zygosity. In a recent review of clinical twin studies of ADHD, each study reported greater concordance rates in MZ twins, relative to DZ twins (Mueller & Tomblin, 2012). The weighted average proband-wise concordance rate was estimated at 71% for MZ twins, and 41% for DZ twins (Mueller & Tomblin, 2012). The clinical twin studies of ADHD underline that ADHD is heritable, and taken together with family studies underline that ADHD familiarity is largely due to genetic factors.

1.3.2 Overview of the twin method

Twins provide a naturally occurring quasi-experimental comparison and are ideal to disentangle genetic and environmental influences underlying phenotypic variation, leading to the wide establishment of large population-based twin registers. The underlying premise of the classical twin design is based on the different genetic relatedness between twin pairs and the assumption that the extent of shared environment does not differ according to zygosity (termed the ‘equal environments assumption’). (See section 1.3.2.6 for a selective overview of limitations of the twin design, and section 7.4.1 for a brief discussion of these limitations, with particular reference to ADHD symptoms).

MZ twins are genetically identical (sharing 100% of all their genes), whereas DZ twins share, on average, 50% of their additive genes and 25% of their dominant genes. As previously mentioned, the main assumption of twin studies is that the extent of shared environment is the same across zygosity. Therefore, greater within-pair phenotypic resemblance in MZ pairs compared to DZ pairs, implicate genetic influences underlying trait variation. Moreover, DZ similarity that is less than half of MZ similarity, infer dominant genetic effects. Additive genetic effects refer to the sum of genetic effects across multiple loci, and dominant genetic effects refer to the interactive genetic effects at the same loci. The sum of additive and dominant genetic effects can be referred to as broad-sense heritability, whereas additive genetic effects are commonly referred to as heritability. Phenotypic dissimilarity in MZ twins is attributed to non-shared environmental influences (environmental factors that contribute to differences in

children within the same family). If DZ similarity exceeds half of MZ resemblance, shared environmental influences (factors that contribute to similarities in children within the same family) are indicated. Sibling interaction effects (where the behavioural rating of one twin affects the behavioural rating of their co-twin (see section 1.3.7.2)) mimic dominant genetic effects, by displaying a similar correlational pattern (DZ similarity less than half of MZ similarity). However, both dominant genetic and sibling interaction effects can be further distinguished on examination of variance differences by zygosity, as sibling interaction additionally contributes to significant variance differences as a function of zygosity.

1.3.2.1 The classical twin design

The underlying logic of the classical twin design is that differences in the genetic relatedness between MZ twins, who share all their genetic variation, and DZ twins, who share, on average, 50% of their additive genetic and 25% of their dominant genetic variance, allow phenotypic variance to be decomposed into genetic and environmental components. Based on this difference in genetic relatedness and the assumption that the extent of shared environment does not differ according to zygosity, the relative contribution of additive genetic effects (additive effects of genes at multiple loci (A)), dominant genetic effects (interactive genetic effects at a single loci (D)), shared environmental effects (environmental factors that serve to make twins more similar (C)), and non-shared environmental effects (environmental factors that contribute to twin dissimilarity (E)) can be estimated. As E also includes measurement error it cannot be dropped from models.

With samples consisting of only twins reared together there are three observed statistics (phenotypic variance, MZ covariance, and DZ covariance), which is insufficient to estimate four latent parameters (A, C, D, and E) (as the number of observed statistics must be greater than the number of estimated parameters, due to constraints of model identification). Although aetiologically plausible, in samples of only twins reared together C and D can only be estimated separately, as the effects of C and D are confounded (C will decrease differences between MZ and DZ correlations, whereas D will increase differences) (Neale & Cardon, 1992; Polderman et al., 2007). The selection of which parameters (C or D) to model is determined by the pattern of similarity across zygosity (see section 1.3.2). Including data from additional groups, such as parents of twin pairs, will provide additional information to simultaneously estimate the effects of C and D in the same model.

Twin correlations (r_{MZ} , r_{DZ}) provide an index of twin similarity, and simple equations that use twin correlations can provide a preliminary estimate of aetiological components of phenotypic variance. As MZ twins are, on average, twice as similar as DZ twins in terms of additive genetic sharing, A can be estimated as $2(r_{MZ} - r_{DZ})$. As shared environmental influences serve to make MZ twin resemblance greater than the effects of genetics alone, C can be estimated as $r_{MZ} - A$. Non-shared environmental factors are indicated where MZ similarity is less than unity, so E is estimated as $1 - r_{MZ}$.

Twin correlations can also be used to obtain an initial impression of the presence of aetiological sex differences. If DZ opposite-sex twin correlations are significantly different than DZ same-sex twin correlations, this is indicative of qualitative sex differences (gender difference in specific genetic and environmental influences). If twin correlations differ for males and females, this is indicative of quantitative sex differences (gender differences in the magnitude of aetiological contributions to trait variation).

Although twin correlations are useful to provide a preliminary impression of aetiological contributions to trait variation, formal structural equation modelling allows more accurate parameter estimations to be generated, yields confidence intervals for parameter estimates, can model additional parameters such as sibling interaction, formally tests for aetiological sex differences (see section 3.3.3.3 for more details), and assesses the fit of alternative models. Structural equation models are often represented in terms of path diagrams (see section 1.3.2.2).

1.3.2.2 Path diagrams

Path diagrams are a graphical display of the relationship between observed (measured) and latent (unmeasured) factors (see Figure 1.1). Observed variables are represented as rectangles and latent variables as circles, and the covariance of latent factors is fixed to 1. Single-headed arrows reflect directional pathways, and the influence of A, C, D, and E on phenotypic variation are reflected by regression coefficients a , c , d , and e . Double-headed arrows reflect correlational pathways. As MZ twin pairs share all their genes, and DZ pairs share, on average, 50% of their additive genes, additive genetic correlations (r_A) are 1.0 and 0.5, respectively. Non-shared environment is by definition uncorrelated between twin pairs, regardless of zygosity. Shared environmental correlations (r_C) do not differ by zygosity, and are estimated at 1.0 (see top model of Figure 1.1). Dominant genetic effects involve the interaction between two alleles. In order for D to contribute to the covariance between relatives, they need to share the same two alleles. In the ADE model (see middle model of Figure 1.1) the dominant genetic correlation (r_D) is set to 1.0 for MZ twins as MZs are genetically identical; and 0.25 for DZ pairs, as on average (like full siblings), they share the same two alleles only 25% of the time. The pattern of observed twin correlations will indicate whether an ACE or ADE model should be fitted (see section 1.3.2). A bi-directional sibling interaction pathway (i) can also be included, to model when the behavioural rating of one twin affects the behavioural rating of their co-twin. In ADHD, this sibling interaction effect is negative, so that higher symptom scores in one twin correspond to lower scores in their co-twin (see bottom model of Figure 1.1).

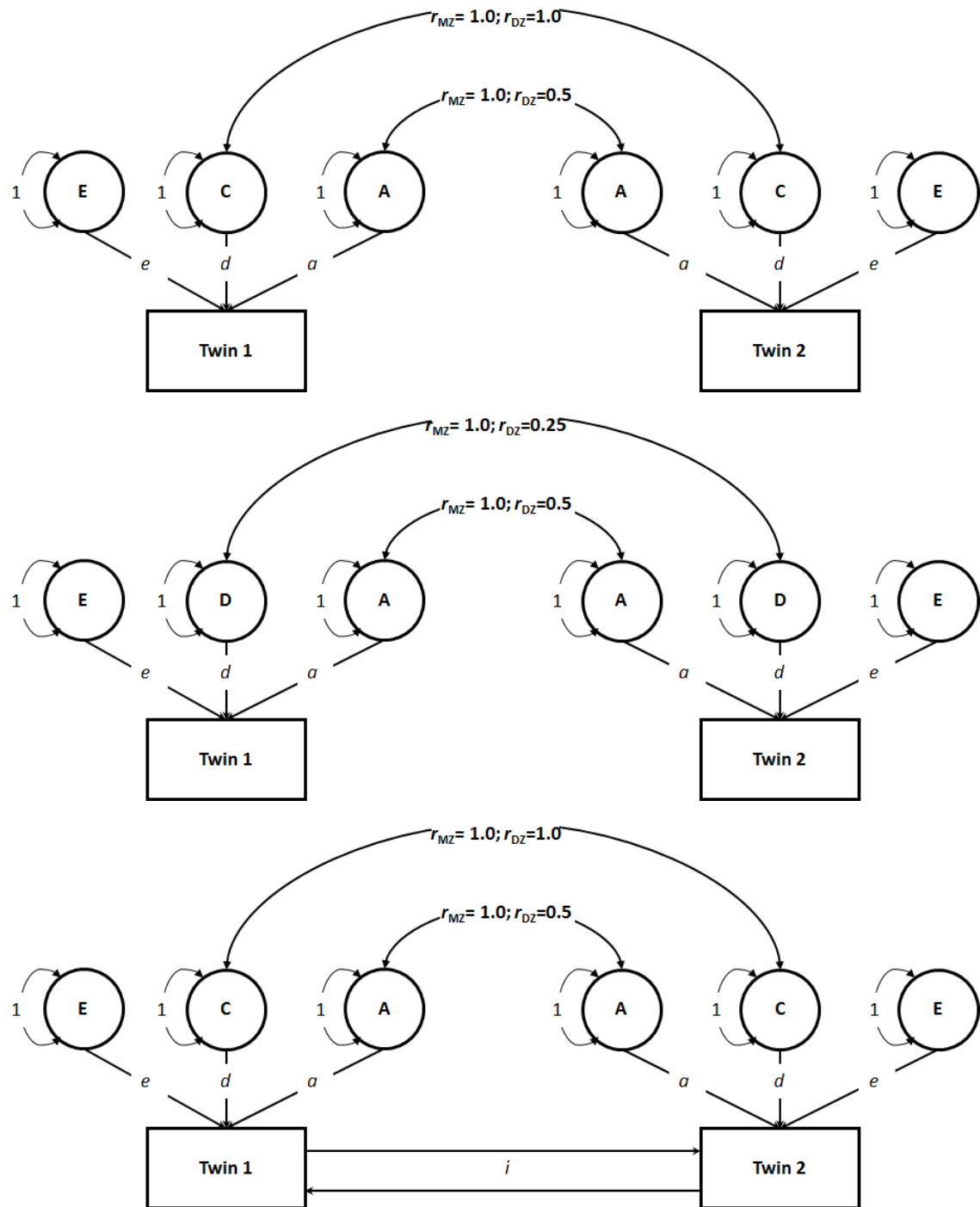
Path-tracing can be used to calculate variances and covariances. Some simple rules apply: for each pathway you cannot trace forwards and then backwards, but can trace backwards and then forwards; each path can pass through only one latent factor and along only one double-headed arrow.

The variance of a trait is the covariance of a trait with itself. For example, additive genetic variance for each twin can be estimated by tracing backwards on the directional path a , through the correlational path of latent factor A (fixed to 1), and then forwards on path a .

These coefficients are multiplied, resulting in additive genetic variance for a trait estimated as a^2 . The same approach can be followed for dominant genetic (d^2) and environmental variances (c^2 and e^2). The sum of these squared factor loadings make up total phenotypic variance. So in the ACE model total phenotypic variance is $a^2 + c^2 + e^2$; in the ADE model total phenotypic variance is $a^2 + d^2 + e^2$.

The covariance between twin pairs can be calculated by tracing paths that connect one twin with their co-twin. For MZ twins, additive and dominant genetic covariance can be estimated as $a*1*a = a^2$ and $d*1*d = d^2$, respectively. For DZ twins, additive and dominant genetic covariance is respectively calculated as $a*0.5*a = 0.5a^2$ and $d*0.25*d = 0.25d^2$. Shared environmental covariance for both MZ and DZ twins is estimated as $c*1*c = c^2$. Thus, total phenotypic covariance for MZ twins in the ACE model is a^2+c^2 ; in the ADE model total phenotypic covariance for MZ twins is $a^2 + d^2$. Total phenotypic covariance for DZ twins in the ACE model is $0.5a^2 + c^2$; in the ADE model total phenotypic covariance for DZ twins is $0.5a^2 + 0.25d^2$.

Figure 1.1 The three classical univariate twin models



Note: upper figure: ACE model; middle figure: ADE model; lower figure: ACEi model; covariance of each latent factor is fixed to 1 (and will be omitted from path diagrams throughout the remainder of this thesis for ease of presentation).

1.3.2.3 Multivariate genetic analysis

In addition to decomposing variance of a single phenotype into genetic and environmental components (univariate analysis), genetically informative samples can be used to assess the aetiological sources of covariation between two or more phenotypes (multivariate analysis). Multivariate genetic analyses not only estimate the underlying aetiology of the covariation between traits, but also the aetiological components of individual trait variation. Multivariate genetic analyses afford greater power than univariate genetic analyses (Schmitz, Cherny, & Fulker, 1998), by decreasing the rate of false positive (type I) error rates (reducing multiple testing). Accordingly, in this thesis when investigating multiple traits, only parameter estimates from multivariate analyses are presented.

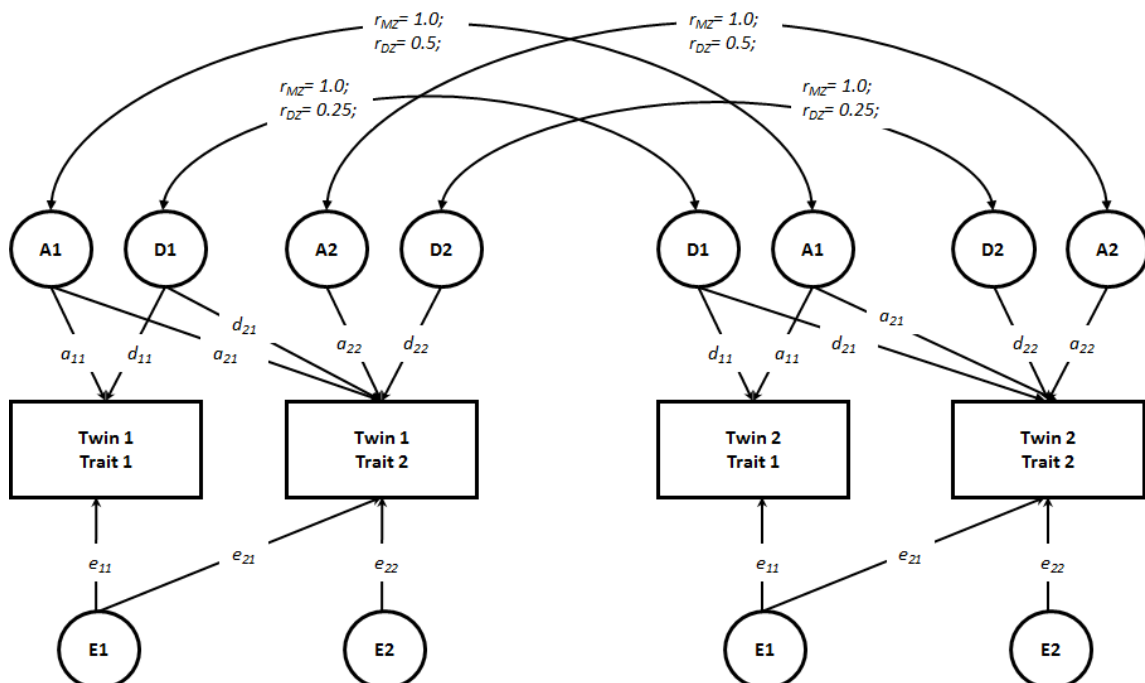
MZ and DZ cross-twin cross-trait (CTCT) correlations are the key informative source of multivariate twin studies (Posthuma, 2009). CTCT correlations refer to correlations within twin pairs across two traits (i.e. correlation between trait 1 of one twin and trait 2 of their co-twin) and provide a preliminary impression of genetic and environmental trait covariation. As the magnitude of CTCT correlations is limited by the phenotypic correlation between the two traits investigated, the degree to which MZ CTCT correlation deviates from the phenotypic correlation implicates non-shared environmental factors as contributing to the covariation between two traits. A larger MZ CTCT correlation, versus DZ CTCT correlation, implicates genetic contributions to trait covariation, with DZ CTCT correlations less than half of MZ CTCT correlations indicative of dominant genetic effects. DZ CTCT correlations greater than half the MZ CTCT correlation implicate that the covariation between two traits is due to shared environmental factors.

The multivariate genetic approaches employed in this thesis include the Cholesky Decomposition and the mathematically equivalent correlated factors solution (Loehlin, 1996). See sections 1.3.2.4 and 1.3.2.5 for more details.

1.3.2.4 The Cholesky Decomposition

Figure 1.2 is a bivariate Cholesky ADE Decomposition, which can be extended to include more than two traits. Genetic and environmental sources that contribute to phenotypic variance in trait 1 and to phenotypic variance shared between traits 1 and 2 are indicated by A1, D1, and E1. Aetiological influences specific to trait 2 (independent of effects shared with trait 1) are indicated by A2, D2, and E2. Therefore, the first set of latent aetiological factors (A1, D1 and E1) can affect all traits, but the second set (A2, D2 and E2) can only affect the second trait. If more than two traits are modelled, the first set of aetiological latent factors can contribute to all traits; the second set of aetiological latent factors can contribute to the second and all further traits; the third set can contribute to the third (but not the first or second) and all subsequent traits (a similar pattern is repeated for all subsequent traits). Therefore, the Cholesky Decomposition gives the first set of latent factors precedence over the others (can affect all measured variables), and therefore the ordering of variables is important. Consequently, the Cholesky Decomposition is utilised where the specific ordering of variables is justified (such as with longitudinal measurements).

Figure 1.2 Bivariate Cholesky ADE Decomposition



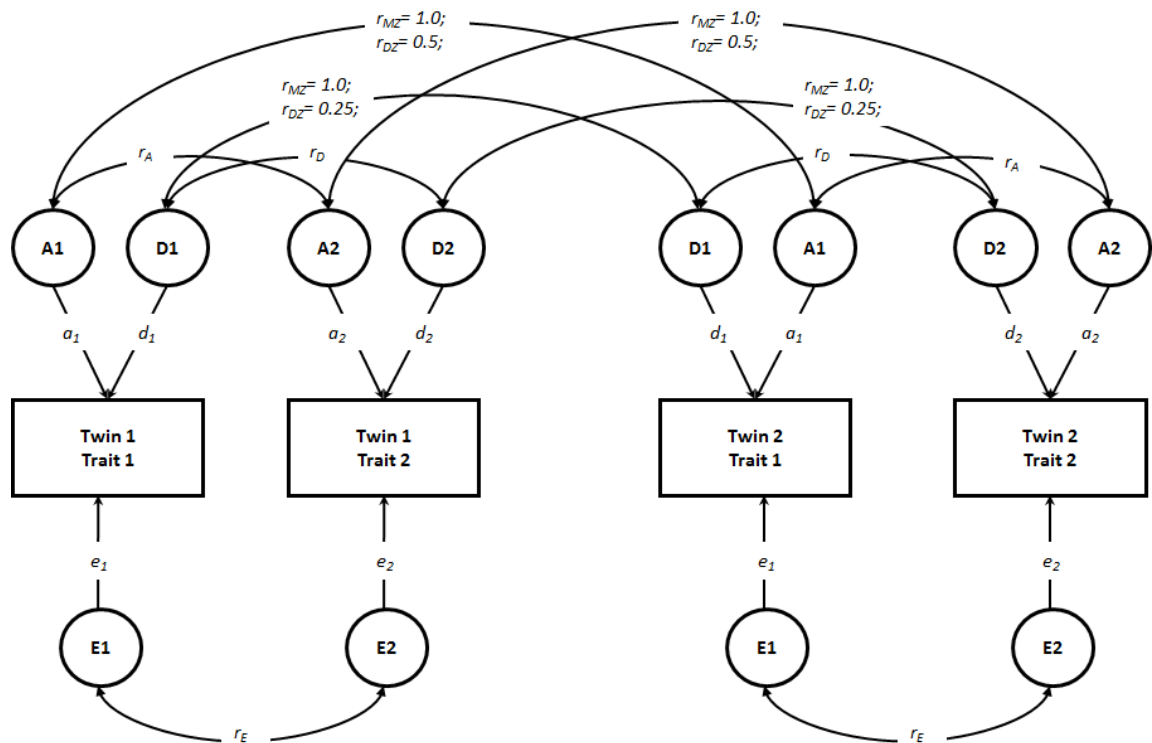
1.3.2.5 Correlated factors solution of the Cholesky Decomposition

As already stated, the ordering of variables is important in the Cholesky Decomposition. However, when the order of variables is arbitrary, the Cholesky Decomposition can be converted into a mathematically equivalent correlated factors solution (Loehlin, 1996); (see Figure 1.3 for the correlated factors solution of the ADE bivariate Cholesky Decomposition). In the correlated factors solution each trait is influenced by genetic and environmental factors estimated by squaring the path coefficients (a_1 , a_2 , d_1 , d_2 , e_1 , and e_2). Thus the phenotypic variance for trait 1 = $a_1^2 + d_1^2 + e_1^2$, and for trait 2 = $a_2^2 + d_2^2 + e_2^2$.

In the correlated factors solution latent aetiological factors are allowed to correlate. The correlation between two factors (e.g., A_1 and A_2) is the additive genetic correlation (r_A) between variables 1 and 2, indicating the proportion of additive genetic factors overlapping for both traits (r_A of 1 implicates completely overlapping additive genetic factors; r_A of 0 implicates no overlapping additive genetic influences). The correlation is independent of the heritability of the two phenotypes (i.e. heritability of both traits could be high and have a low genetic correlation, or vice-versa). Similar correlations can be modelled for dominant genetic (r_D), and both environmental influences (r_C and r_E).

The phenotypic correlation (r_{PH}) between trait 1 and trait 2 is the sum of all genetic and environmental contributions ($(a_1 * r_A * a_2) + (d_1 * r_D * d_2) + (e_1 * r_E * e_2)$). The proportion of the phenotypic correlation between two variables due to additive genetic effects (bivariate additive heritability), is the product of the two additive genetic path coefficients and the additive genetic correlation between these two traits, divided by the total phenotypic correlation $((a_1 * r_A * a_2) / r_{PH})$. Using the same principles the same estimates can be obtained for dominant genetic, and both environmental factors.

Figure 1.3 Bivariate correlated factors solution of the ADE Cholesky Decomposition



1.3.2.6 Limitations of the twin method

Equal environments assumption: One of the main assumptions of the twin method is that the extent of environmental influences shared between twin pairs does not differ as a function of zygosity, termed the equal environments assumption. Violations of this assumption (e.g., if twin pairs are treated more alike or exposed to more similar environments, according to zygosity) can impact parameter estimates. Specifically, MZ correlations would be increased if MZ twins experience more similar environments than DZ twin pairs, leading to an overestimation of genetic influences. The effect of the equal environments assumption may work in the opposite direction, where shared environmental similarity is greater for DZ versus MZ pairs (e.g. due to the systematic separation of MZ twins into different classrooms, compared to DZ pairs), which would lead to increased DZ correlations, contributing to an overestimation of shared environmental influences (Rijsdijk & Sham, 2002).

Assortative mating: Assortative mating arises when mate selection is not entirely random. The effect of positive assortative mating ('birds of a feather flock together') contributes to greater DZ twin pair correlations (as the average genetic similarity of DZ twins is increased, whereas MZ genetic similarity is already 100%), leading to reduced heritability estimates.

Generalisability: Another issue related to the twin method is whether twins can be considered as representative of the general population, and as such can findings from twin samples generalise to singletons. There is evidence to suggest that compared to singletons, twin pairs tend to have lower birth weight, are more frequently associated with obstetric and pregnancy complications and born more prematurely (Rijsdijk & Sham, 2002). Moreover, twins tend to show delays in language attainment and cognitive ability, although this group difference is absent by middle childhood (Plomin et al., 2008).

See section 7.4.1 for brief review of these limitations in relation to ADHD symptoms.

1.3.3 ADHD as a quantitative trait

Population-based twin samples avoid sample ascertainment biases inherently associated with selected or clinic-referred samples. The vast majority of ADHD twin studies have utilised questionnaire-based rating scales to measure ADHD behaviours (Wood & Neale, 2010). Instead of applying a cut-off for group membership, total symptom counts are employed so that ADHD behaviours are viewed along a severity continuum. Although such a dimensional approach is not equivalent to a clinical diagnosis (requiring endorsement of additional criteria such as age of onset, symptom duration, functional impairment, and situational pervasiveness), rating scales facilitate the collection of phenotypic data for large samples, while limiting economic and time-related costs (Derks et al., 2008). Moreover, there is substantial converging evidence from quantitative genetic analyses to corroborate a dimensional view of ADHD genetic liability.

The majority of ADHD twin studies have defined ADHD as a continuous trait, and pooled twin studies of ADHD symptoms have yielded heritability estimates ranging from 0.62 to 0.76 (Biederman & Faraone, 2005; Burt, 2009; Wood, Buitelaar, Rijdsdijk, Asherson, & Kuntsi, 2010). Twin studies of categorical classes based on parental ratings have yielded comparable heritability estimates (0.73 to 0.85) (Kuntsi et al., 2004; Sherman, McGue, & Iacono, 1997; Thapar, Harrington, Ross, & McGuffin, 2000), supporting the use of quantitative measures of ADHD behaviours.

A number of twin studies have adopted the DeFries and Fulker (1985) multiple regression approach to estimate group heritability, derived from the extent to which co-twins of MZ probands (extreme scoring individuals) have mean scores more similar to their proband, than co-twins of DZ probands. Significant group heritability implicates genetic continuation between high and low ADHD scores, and between normal variation and at the extreme. Reported extreme group heritability estimates are in line with those observed for normal trait variation in the general population, ranging from 0.83 to 0.93, and relatively consistent regardless of severity cut-off applied (Levy, Hay, McStephen, Wood, & Waldman, 1997; Price, Simonoff, Waldman, Asherson, & Plomin, 2001). Furthermore, a direct comparison of ADHD dimensional and extreme group heritability reported no significant differences (Levy et al., 1997).

The first study to apply the multiple regression approach to DSM-IV based ADHD ratings (Larsson, Anckarsater, Rastam, Chang, & Lichtenstein, 2012), confirmed strong, but slightly lower, group heritability estimates across two defined cut-off points (0.60 and 0.62), in a large sample of over 8000 twin pairs aged nine and 12. A similar pattern was found when examining inattention and hyperactivity-impulsivity separately, with group heritability estimates of 0.53 and 0.62, respectively (Larsson et al., 2012).

Although some of the above studies employed cut-offs in line with diagnostic assessments, further differences between the diagnostic and continuum definitions include additional diagnostic criteria (e.g., age of onset, functional impairment, symptom persistence, and observation across multiple settings). A study addressing this gap by using a clinical sample of probands and their siblings, reported moderate familial correlations between probands with DSM-IV ADHD-C subtype and their sibling's DSM-IV based quantitative ADHD symptom score (0.21 for parental and 0.30 for teacher ratings) (Chen et al., 2008), providing additional support for a dimensional view of ADHD behaviours.

Further support for a dimensional approach was obtained from a study comparing latent class analysis (which assumes qualitative (subtype) differences) and factor analysis (which assumes quantitative (severity) differences) of maternal ADHD ratings for male same-sex twins (Lubke, Hudziak, Derks, van Bijsterveldt, & Boomsma, 2009). Factor mixture modelling which simultaneously combines both approaches, revealed the best fitting model to be one that distinguished classes based on severity (mild, moderate and severe) (Lubke et al., 2009). Subsequent analysis on a subsample with diagnostic information, found that no children with DSM-IV ADHD were classified in the mildly severe group (Lubke et al., 2009), underlining ADHD as representing the extreme of a continuous severity distribution of ADHD symptoms.

Taken altogether, the evidence suggests that ADHD genetic liability operates dimensionally: similarly influencing normal variation in the general population, as well as extreme scores, and that ADHD can be considered as lying at the tail end of a continuous dimension. This has important implications for ADHD genetic research, as it suggests that findings derived from quantitative measures of ADHD may generalise to clinical ADHD. As already outlined, employing rating scales in population-based samples allow the gathering of large amounts of

data, thereby increasing statistical power for quantitative and molecular genetic investigations. This thesis adopts a dimensional view of ADHD, and the majority of the analyses in this thesis utilises quantitative measures of ADHD behaviours in population-based samples.

1.3.4 The genetic and environmental aetiology of ADHD symptoms

1.3.4.1 The genetic aetiology of ADHD symptoms

Pooled twin studies of ADHD symptoms have yielded broad-sense heritability estimates ranging from 62% to 76% (Biederman & Faraone, 2005; Burt, 2009; Wood, Buitelaar et al., 2010). In a meta-analysis of aetiological studies of childhood psychopathologies (Burt, 2009), the predominance of dominant versus additive genetic effects (44% and 26%, respectively), and the negligible influence of shared environmental factors (see section 1.3.4.2), were cited as distinguishing features of ADHD aetiology (Burt, 2009). Moreover, the findings revealed substantial stability of the magnitude of broad-sense heritability, but a developmental decline in dominant genetic effects, comprising a significantly larger component of variation in ADHD ratings in the youngest (up to age five) versus older (aged six to 11, and 12 to 18) samples (Burt, 2009). Broad-sense heritability for ADHD ranged from 58% to 73% when examining potential moderators of heritability estimates (including age and gender of rated children, assessment instruments and informant) (Burt, 2009). The only exception was for self-reported ADHD ratings, which yielded much lower (36%) broad-sense heritability estimates, with the majority of the variance attributed to non-shared environmental influences (Burt, 2009). Research suggests that this is likely to be an artefact of informant (see section 1.3.7.3).

Similarly high broad-sense heritability estimates were reported in a meta-analysis of inattentive (71%) and hyperactivity-impulsivity (73%) symptom domains (Nikolas & Burt, 2010). Although the genetic contribution for both behavioural dimensions reflect predominantly additive genetic effects, this component was significantly larger for hyperactivity-impulsivity (71%) versus inattention (56%), whilst dominant genetic effects were significantly larger for inattention (15%) versus hyperactivity-impulsivity (2%) (Nikolas & Burt, 2010). Compared to hyperactivity-impulsivity the magnitude of aetiological components for inattention were largely consistent throughout development (Nikolas & Burt, 2010).

Although explored less extensively than childhood ADHD, adult ADHD heritability estimates are comparatively more moderate, ranging from 30% to 40% (Boomsma et al., 2010; Distel et al., 2011; van den Berg, Willemsen, de Geus, & Boomsma, 2006). Although initially considered as indicative of a developmental decline in ADHD heritability, more recent research suggests that ratings from multiple informants (i.e. self-report ratings (which are frequently used in adult samples)) tend to yield lower heritability, due to increased unreliability associated with ratings from more than one informant, which in effect places a ceiling limit on heritability estimates. Accordingly, the lower heritability estimates observed for adult ratings is likely an artefact of informant effects (see section 1.3.7.3 for more details).

1.3.4.2 The environmental aetiology of ADHD symptoms

As previously mentioned, a meta-analysis of childhood problem behaviours found negligible significant shared environmental effects for ADHD symptoms (Burt, 2009). In contrast, significant shared environmental effects were found for symptoms relating to CD, ODD, depression and anxiety (Burt, 2009). Thus, the absence of any meaningful effect of shared environmental factors was proposed as a distinctive feature of ADHD aetiology (Burt, 2009). However, a subsequent comment to this meta-analysis contested that a number of methodological issues inherent to twin studies, particularly the confounding effects of modelling C and D, have contributed to the misconception that shared environmental factors do not constitute a significant aetiological component of ADHD behaviours (Wood, Buitelaar et al., 2010). However, subsequent analysis on a sample of adopted siblings (which avoids the confounding effects of D and C, as only C is estimated) by the author of the original meta-analysis, found no significant effect of C, indicating that the absence in twin studies is not due to the confounding effects of D (Burt, 2010). More recently the authors of the original meta-analysis used the extended twin design (including data from parents of twin pairs, which allows the estimation of both D and C), and similarly found no significant shared environmental influences (Burt, Larsson, Lichtenstein, & Klump, 2012). The authors concluded that further research attention should be directed at identifying specific non-shared environmental factors which contribute to the majority of the non-genetic variance of ADHD (Burt et al., 2012). However, it is important to note that non-shared environmental factors subsume

measurement error, and accordingly pure idiosyncratic environmental effects likely account for less variance than is currently assumed by quantitative genetic studies.

The most promising approach to identify non-shared environmental components is to utilise the MZ discordant approach (Ronald & Hoekstra, 2011), where disorder discordance in MZ twins must be attributed to non-shared environmental sources, as MZs are genetically identical. However, samples of MZ ADHD discordant twins are typically small, due to ADHD being under considerable genetic influence. In a study examining the contribution of specific non-shared environmental factors to ADHD presentation, differences in sibling interaction, parental treatment, and peer relations, but not specific life events, were reported in sibling pairs discordant for ADHD. Moreover, these differential experiences were not only associated with differences in the severity of ADHD symptoms, but also to differences in comorbid behaviours (Buschgens et al., 2008). Although this study identified specific sources of non-shared environmental factors within ADHD discordant siblings, one cannot be certain that these were not confounded by genotype differences within sib-pairs.

1.3.5 Longitudinal studies

Longitudinal studies have reported moderately high stability of ADHD behavioural ratings within early childhood (Ilott, Saudino, & Asherson, 2010; Kuntsi, Rijdsdijk, Ronald, Asherson, & Plomin, 2005; Price et al., 2005), and from middle childhood to early adolescence (Greven, Asherson, Rijdsdijk, & Plomin, 2011; Larsson, Larsson, & Lichtenstein, 2004). Self-reported ratings of the attention problem (AP) subscale in the Young Adult Self-Report scale similarly display moderate stability from late adolescence to young adulthood (van den Berg et al., 2006).

Two longitudinal studies with independent samples of preschool twins reported significant age-specific genetic effects, but that the majority of genetic propensity was shared across ages (Ilott, Saudino, & Asherson, 2010; Price et al., 2005). Studies from early to middle childhood yielded similar patterns, with stability of parental ADHD-related ratings largely ascribed to enduring genetic influences, but with new genetic effects emerging across development (Kuntsi, Rijdsdijk et al., 2005; Rietveld, Hudziak, Bartels, van Beijsterveldt, & Boomsma, 2004). In

a similar vein, enduring genetic factors primarily contributed to the stability of AP ratings over a period of six years from late adolescence to young adulthood (van den Berg et al., 2006).

In sum, ADHD symptoms show substantial phenotypic stability. Accordingly, early detection and increased understanding of the aetiological mechanisms underlying ADHD symptoms is paramount (Polderman, Posthuma, De Sonneville, Verhulst, & Boomsma, 2006). ADHD phenotypic stability in childhood is largely driven by a substantial set of common genetic influences. The substantial enduring genetic propensity within childhood suggests that it may be relatively difficult to identify developmentally-specific versus age-persistent genetic loci (Kuntsi, Rijdsdijk et al., 2005). There is a paucity of studies examining genetic effects on stability and change in ADHD symptoms beyond late adolescence; however, deconstructing such samples may lead to decreased statistical power. Extracting the common variance across different assessment points reduces measurement error (Kuntsi, Rijdsdijk et al., 2005) and captures a more heritability construct (van den Berg et al., 2006), avoiding the need to stratify samples and providing a more genetically influenced target for molecular genetic investigations.

1.3.6 Aetiological sex differences

Cross-sectional studies on the large nationally representative UK-based Twins' Early Development Study (TEDS) have found no evidence of quantitative sex (gender differences in the magnitude of aetiological contributions to trait variation), or qualitative sex differences (gender difference in specific genetic and environmental influences), in parental ADHD ratings during early childhood (Price et al., 2001), middle childhood (Kuntsi, Rijdsdijk et al., 2005; McLoughlin, Ronald, Kuntsi, Asherson, & Plomin, 2007; Merwood, Greven, Larsson, Price, & Asherson, Submitted), or early adolescence (Greven, Rijdsdijk, & Plomin, 2011).

However, a study of TEDS children at age seven reported qualitative sex differences for twins rated by the same teacher (not found for different teacher or parental ratings) (Saudino, Ronald, & Plomin, 2005). Although qualitative sex difference were found for teacher ratings in an independent twin sample (Derks, Dolan, Hudziak, Neale, & Boomsma, 2007), subsequent

analysis for ratings at age 12 in TEDS found no sex differences in either same or different teacher ratings (Merwood et al., Submitted).

Taken together there is no evidence of aetiological sex difference in parental ADHD ratings, and accordingly molecular genetic investigations of parental ratings need not stratify samples by gender. There is limited evidence that teacher ratings of girls and boys are influenced by partly different genetic factors. If qualitative sex differences are verified, then molecular genetic investigations based on teacher ratings should stratify by gender as sex-specific genetic variation will be obscured by samples including both males and females (Derks et al., 2007). However, until these findings are replicated they should be treated with caution, as the majority of evidence suggests that there are no significant aetiological sex differences in ADHD ratings (Thapar & Stergiakouli, 2008).

1.3.7 Informant effects

1.3.7.1 Informant effects: parent versus teachers

Studies examining heritability across informants have generally reported similar heritability estimates for maternal and paternal ADHD ratings (Eaves et al., 1997; van Beijsterveldt, Verhulst, Molenaar, & Boomsma, 2004). In contrast, there is a tendency for lower heritability estimates for teacher versus parental ratings (Derks, Hudziak, Van Beijsterveldt, Dolan, & Boomsma, 2006; Kuntsi & Stevenson, 2001; Sherman, Iacono, & McGue, 1997; Thapar et al., 2000; Vierikko, Pulkkinen, Kaprio, & Rose, 2004; Wood, Rijdsdijk, Saudino, Asherson, & Kuntsi, 2008), although this has not been universally found (Martin, Scourfield, & McGuffin, 2002; Polderman et al., 2006). One potential explanation for the observed discrepancy according to informants may stem from biases specific to parents, which artificially increase differences between MZ and DZ correlations and thereby contribute to inflated heritability estimates.

1.3.7.2 Low DZ correlations: rater contrast effects

A relatively consistent finding in twin studies of parental ADHD ratings is that DZ correlations are much smaller than half of MZ correlations (Kuntsi, Gayan, & Stevenson, 2000; Kuntsi & Stevenson, 2001; Martin et al., 2002; Saudino et al., 2005; Sherman, Iacono et al., 1997; Thapar

et al., 2000; Vierikko et al., 2004; Wood et al., 2008). Low DZ correlations are indicative of the presence of dominant genetic or sibling interactive effects, which can further be distinguished as the latter causes significant variance differences by zygosity (Rietveld, Hudziak, Bartels, van Beijsterveldt, & Boomsma, 2003; Simonoff et al., 1998). However, particularly large samples are required to detect small, but significant, sibling interaction effects (Rietveld, Posthuma, Dolan, & Boomsma, 2003).

Sibling interactions can either be cooperative or competitive. In the case of ADHD parental ratings the relationship is negative, so that the higher the phenotypic rating for one twin, the lower the rating for the co-twin. This phenotypic interaction may arise from either competitive sibling interaction, whereby the behaviour of one twin influences the behaviour of the co-twin, or reflect a form of rater bias where parents emphasise behavioural differences. These hypotheses were tested in a population-sample of twins from the Virginia Twin Study of Adolescent Behavioral Development (VTSABD) (Simonoff et al., 1998) that included a reciprocal sibling interaction pathway between maternal ratings (to reflect rater bias), and between latent hyperactivity phenotypes for twin 1 and twin 2 (to reflect sibling interaction). Model fitting analysis revealed that the latent phenotypic contrast parameter, and not the maternal rater contrast effect, could be dropped, suggesting that contrast effects are a form of rater bias (Simonoff et al., 1998). Teacher ratings were also modelled and reflected two different forms of bias (twin confusion and correlated errors (see section 1.3.7.3)) (Simonoff et al., 1998).

Greater DZ resemblance for mechanically-assessed activity level (Wood, Saudino, Rogers, Asherson, & Kuntsi, 2007) or observer-rated activity level (Saudino, Cherny, & Plomin, 2000), further corroborate the observed sibling interaction in ADHD parental ratings as perceptual. True phenotypic differences would be expected across raters and so additional support is provided by the absence of contrast effects in teacher ratings, despite utilising corresponding measures in the same sample as where parental contrast effects have been observed (Kuntsi et al., 2000; Kuntsi & Stevenson, 2001; Saudino et al., 2005; Vierikko et al., 2004). The absence of such rater contrast effects in teacher ratings is hypothesised to stem from their increased exposure to children and consequently greater expertise in normative behaviours (Simonoff et al., 1998).

A study on parental ratings across a range of subscales from the Strengths and Difficulties Questionnaire (SDQ) found contrast effects for ADHD ratings, but not for pro-social behaviours, peer problems, emotional symptoms, or conduct problems (Saudino et al., 2005). This suggests that parental ratings of ADHD symptoms seem particularly prone to rater contrast effects, hypothesised to stem from a lack of consensus regarding normative ADHD-related behaviours (Saudino et al., 2005).

Rater contrast effects have been found for both maternal and paternal ADHD ratings (Nadder, Silberg, Rutter, Maes, & Eaves, 2001; Saudino et al., 2000; van Beijsterveldt et al., 2004), but are also not universally found in parental ADHD ratings (Greven, Rijdsdijk et al., 2011; Hay, Bennett, Levy, Sergeant, & Swanson, 2007; Hudziak, Derks, Althoff, Rettew, & Boomsma, 2005; Hudziak, Rudiger, Neale, Heath, & Todd, 2000; Levy et al., 1997; Martin et al., 2002; McLoughlin et al., 2007; Polderman et al., 2007; Tuvblad, Zheng, Raine, & Baker, 2009), suggesting biases may be specific to rating scales and/or sampling demographics (Polderman et al., 2007) (see section 2.2 and chapter 2).

Parental contrast effects may have significant implications for sampling strategies for molecular genetic studies (Price et al., 2005; Rietveld, Hudziak et al., 2003; Simonoff et al., 1998). Emphasising behavioural differences may negatively impact the identification of concordant/discordant pairs, contributing to concordant twin pairs being under-represented (false-negative selection) and discordant twin pairs being over-represented (false-positive selection) (Rietveld, Hudziak et al., 2003; Simonoff et al., 1998). Consequently, ratings from scales that are more resistant to rater contrast effects and from multiple informants (as teacher ratings are not gold standard (see section 1.3.7.3)) should be encouraged for genetic investigations.

1.3.7.3 Teacher ratings: same- versus different-teacher ratings

In addition to rater contrast effects in parental ADHD ratings potentially inflating heritability estimates, combining ratings from twins rated by the same and different teachers may also contribute to the lower heritability estimated for teacher ratings (see section 1.3.7.1). In line with this, SDQ ratings in the TEDS sample at age seven yielded lower heritability estimates for

ADHD ratings from different teachers (boys: 66%; girls: 55%), but comparable heritability estimates for ratings from parents (boys: 75%; girls: 77%) and same-teachers (boys: 74%; girls: 76%) (Saudino et al., 2005). Moreover, model fitting analysis that compared same- and different-teacher ADHD ratings reported no significant differences in the magnitude of shared environmental contributions, suggesting that being in the same class (which is likely if rated by the same teacher) does not contribute to increased shared environmental influences (Saudino et al., 2005). However, when non-shared environmental contributions were compared across teacher ratings, significantly larger effects were found for ratings obtained from different- versus same- teachers (Saudino et al., 2005).

A similar pattern was observed in a subsequent study based on the same sample with the same rating scale at age 12 (Merwood et al., Submitted). Univariate analysis revealed similar heritability estimates for parental (77%) and same-teacher ratings (75%), and lower heritability estimates for self-report (48%) and different-teacher ratings (47%) (Merwood et al., Submitted). The authors suggested that reliability across two different raters (in the case of different-teacher and self-report ratings) is likely to be compromised, compared to those obtained from one individual (in the case of same-teacher and parent ratings), due to raters applying different rater biases (styles, tendencies, and normative standards). Accordingly this lower reliability contributes to increased measurement error (subsumed by non-shared environmental variance), and in effect places a ceiling limit on heritability estimates (Merwood et al., Submitted).

Taken together, these findings suggest that the lower heritability estimates reported for adult ADHD (see section 1.3.3), are attributable to informant effects associated with self-report (multiple) ratings, rather than a developmental drop in genetic influences.

Overall, correlations between ADHD ratings for twins from the same teacher are greater than ratings made by different teachers (Derks et al., 2007; Derks et al., 2006; Polderman, Posthuma et al., 2006; Saudino et al., 2005; Simonoff et al., 1998). The higher twin correlations observed for same-teacher ratings may stem from increased shared method variance (referred to as correlated errors). Another explanation may be difficulty ascribing behaviours to the correct child (referred to as twin confusion). In the correlated errors model, E is allowed to correlate in

children rated by the same teacher (r_E), and if significantly greater than zero, is indicative of such a bias (correlated errors). In the twin confusion model, measured variables are modelled to reflect both the individual's and co-twin's behaviour.

Both the correlated errors and twin confusions models were fitted to the VTSABD sample and provided a good fit to the data (Simonoff et al., 1998). Although correlated errors and confusion effects are not mutually exclusive mechanisms, incorporating both did not result in an improved fit to either individual model, although the authors noted there may have been limited power to detect both mechanisms simultaneously (Simonoff et al., 1998). Comparing individual models suggested that the correlated errors model fitted the data better, although the magnitude of the difference in model fit was small (Simonoff et al., 1998). In a separate study on an independent sample of twins aged seven years old, the correlated errors model ($r_E = 0.54$) provided a better fit than the twin confusion model (Derks et al., 2006). A similar estimate ($r_E = 0.49$) was reported for ratings of the same sample at age five (Polderman, Posthuma et al., 2006). Although this study did not test the applicability of the twin confusion model, dropping r_E resulted in a significant worsening in fit indicating that a correlated error bias was significant, and is a potential source of the higher twin correlations observed in children rated by the same versus different teachers.

Taken altogether, findings based on parental and teacher ratings suggest that neither are gold standard. Consequently ratings from multiple informants should be encouraged for genetic investigations.

1.3.7.4 Rater disagreement

Parent and teacher ADHD symptom ratings show low inter-rater agreement (correlating at around 0.3) (Saudino et al., 2005; Thapar et al., 2000). The source of this low correlation may include the situational specificity of raters' interactions (viewing different, but valid, aspects of childrens' behaviour) and/or reflect rater biases (response styles (e.g. leniency), stereotyping tendencies and/or normative standards) (Bartels et al., 2004). These different potential sources of rater disagreement can be disentangled by applying the psychometric model, which assumes that informants assess the same behaviours, in addition to specific aspects of childrens'

behaviour. These rater-specific views in conjunction with rater bias and/or unreliability contribute to the observed inter-rater disagreement (Bartels et al., 2004). Both the common and unique behavioural aspects are allowed to be influenced by genetic and environmental effects, and the assessment of valid behaviours is implicated if the unique component is genetically influenced. Common 'E' reflects only pure idiosyncratic environmental effects, and significant rater-specific C and E parameters respectively implicate rater bias and unreliability as contributing to observed inter-rater disagreement. In contrast to the psychometric model, the rater bias model assumes that informants are assessing the same behaviours, and that inter-rater disagreement is a result of measurement error and/or biases specific to informant.

In a study comparing the fit of the psychometric versus the rater bias model, the former provided a better fit to the data (Hartman, Rhee, Willcutt, & Pennington, 2007), underlining that inter-rater disagreement in ADHD is not entirely driven by rater biases, and that informants rate meaningful, but somewhat different, aspects of children's behaviour.

In an independent twin sample and utilising the psychometric model, both genetically influenced common and rater-specific aspects for parental and teacher ratings were reported (Martin et al., 2002). The common variance across parental and teacher ratings from the Conners' Rating Scale showed moderate shared genetic effects (31%), in addition to rater-specific genetic effects for parent (41%) and teacher (50%) ratings (Martin et al., 2002). Ratings derived from the SDQ showed a common genetic factor (38%) and rater-specific genetic effects (13% and 35% of variance for parent and teacher ratings, respectively) (Martin et al., 2002). Another study found that much of the genetic effects underlying parent rated ADHD also influenced teacher rated ADHD, but that additional specific genetic effects contributed to teacher ratings (Thapar et al., 2000). This pattern was similar whether a categorical or dimensional approach was adopted (Thapar et al., 2000). In a study simultaneously modelling both parent and teacher DSM-IV based inattentive and hyperactivity-impulsivity ratings, a common factor contributed to nearly half of the total variance of hyperactivity-impulsivity ratings by parents, and to a less extent (28%) by teachers (McLoughlin, Rijdsdijk, Asherson, & Kuntsi, 2011). A similar pattern emerged for inattention ratings.

Overall, these studies suggest that both parents and teachers are tapping into the same behaviours; but that informants are also, in part, rating specific aspects of children's behaviour. This may be due to situational effects or because different behaviours are elicited by unique interactions with specific informants. Moreover, while there is shared genetic propensity, genetic effects for rater-specific factors are substantially larger for teacher versus parental ratings. These findings suggest that while parents are rating somewhat similar ADHD behaviours as teachers, teachers are to a greater degree rating a unique perspective. Consequently, parental ratings are likely to contribute to the discovery of common genetic markers, whereas targeting teacher ADHD ratings is likely to yield teacher-specific variants (McLoughlin et al., 2011). Capturing the common variance across raters may facilitate gene-hunting studies. Moreover, the common factor across raters tends to lead to a more heritable construct than individual ratings (Martin et al., 2002; Simonoff et al., 1998; van den Berg et al., 2006), and is likely to reduce measurement error variance (Kuntsi, Rijdsdijk et al., 2005; Simonoff et al., 1998). In addition, compared to ratings obtained from one informant, heritability estimates are greater for combined (summed) parental and teacher ratings (reflective of pervasive ADHD rather than situational ADHD (Thapar, Langley, O'Donovan, & Owen, 2006). For the most part, parental and teacher ratings are summed when examining behavioural ratings in this thesis (chapters 3 to 5).

1.3.8 Assessment instrument effects

An important consideration for both quantitative and molecular genetic studies is whether different ADHD assessment instruments are indexing the same genetic liability. A study that compared interview-based maternal and paternal ADHD measures, and maternal and teacher quantitative rating scale measures, found for both males and females a common genetic factor across raters, assessments, and ADHD symptom domains, in addition to genetic influences specific to interview format and informant (Nadder et al., 2001). For females, there was an additional small genetic factor common to questionnaire ratings (Nadder et al., 2001). In an independent twin study, largely overlapping genetic factors (genetic correlation (r_G) = 0.63 to 0.76) were found for DSM-IV interview and questionnaire measures at age 12 (Derks et al., 2008).

Taken together, these findings suggest that different measurements of ADHD index the same genetic liability, emphasising the appropriateness of using rating scales as an alternative to interview-based data.

1.3.9 Genetic overlap between inattention and hyperactivity symptom domains

Studies examining the genetic overlap of the two separate ADHD symptom domains suggest substantial shared genetic factors, in addition to some genetic specificity, with genetic correlations ranging from 0.55 to 0.83 (Greven, Rijdsdijk et al., 2011; Haberstick et al., 2008; McLoughlin et al., 2011; McLoughlin et al., 2007; Schultz, Rabi, Faraone, Kremen, & Lyons, 2006; Wood, Rijdsdijk, Asherson, & Kuntsi, 2009). Simultaneously modelling both parent and teacher DSM-IV based inattentive and hyperactivity-impulsivity ratings, both symptom domains loaded onto separate latent factors shared across informants ($r_G = 0.74$) (McLoughlin et al., 2011).

Despite the substantial shared genetic component between the two ADHD symptom domains, converging evidence highlights the importance of the partially distinct aetiologies. For example, reading disability has a stronger phenotypic association with inattention (versus hyperactivity-impulsivity), which is largely attributed to overlapping genes (Greven, Harlaar, Dale, & Plomin, 2011; Paloyelis, Rijdsdijk, Wood, Asherson, & Kuntsi, 2010; Willcutt, Pennington, Olson, & DeFries, 2007); whilst oppositional behaviours, in middle childhood, show a higher phenotypic covariation with hyperactivity-impulsivity, with nearly completely overlapping aetiological influences between the two traits (Wood et al., 2009).

The genetic heterogeneity provides further support for multiple neurobiological processes underlying ADHD behaviours, and encourages the separation of these behavioural dimensions for quantitative genetic analysis (chapters 3 and 5).

Taken together, these findings suggest that molecular genetic studies may benefit from targeting inattention and hyperactivity-impulsivity symptom ratings separately. Accordingly in this thesis when investigating the molecular genetic correlates of ADHD behavioural ratings, inattention and hyperactivity-impulsivity are considered separately (chapter 4). Although the

substantial genetic overlap suggests that genes associated with one behavioural dimension are likely to be associated with the other, such an approach may also capture domain-specific genetic variants reflecting the moderate genetic heterogeneity of these two symptom subscales.

1.3.10 Summary of ADHD twin studies

As reflected in the sections above, the utility of quantitative genetic studies transcends beyond merely estimating heritability (Asherson, Kuntsi, & Taylor, 2005). In particular, quantitative genetic studies are useful to guide molecular genetic sampling strategies and inform target selection. Quantitative genetic studies can additionally identify behavioural traits that share overlapping genetic influences, even across diagnostic boundaries, which can guide the selection of additional genetic markers for candidate gene association studies. For example, if an autism spectrum disorder (ASD) and ADHD are found to share a high proportion of overlapping genes in quantitative genetic studies, molecular genetic investigations may benefit from examining putative ADHD risk markers for ASD, and vice-versa.

In sum, ADHD symptoms in childhood and adolescence are highly heritable and longitudinal studies suggest that stability in ADHD symptoms prior to adulthood is largely attributed to genetic effects. Despite rater- and assessment-specific effects, overall parents and teacher ratings largely index the same genetically-influenced behaviours. Both inattention and hyperactivity-impulsivity symptom domains show substantial genetic overlap, in addition to some degree of genetic distinction. The high heritability of ADHD symptoms is remarkably consistent, despite different assessment instruments, sample populations, informants, and whether viewed as a dichotomous category or a quantitative trait. Accordingly, there have been concerted efforts at identifying the specific genetic variants contributing to the large inherited component of ADHD behaviours.

1.4 ADHD aetiology

1.4.1 Genetic risk factors

Despite the established strong genetic component of ADHD and considerable gains in molecular genetic strategies, much of the genetic variance of ADHD remains unaccounted for

(Kuntsi, Neale et al., 2006; Plomp et al., 2009; Purper-Ouakil et al., 2011). In addition, the majority of existing linkage and candidate genes association studies are characterised by conflicting findings (Furman, 2008; Grigorenko, 2012; Martin et al., 2008), a pattern of results often found for complex traits and disorders (Bishop, 2009; Waldman & Gizer, 2006). Accordingly, in this overview of molecular genetic studies we limit discussions to findings yielded from meta-analyses. Although meta-analyses may have resolved some of the discrepant findings from individual studies (Doyle, Faraone et al., 2005), caution is warranted as these meta-analyses are based predominantly on the published literature and there is a likely publication bias against studies reporting negative findings.

1.4.1.1 Linkage studies

Linkage studies are concerned with identifying regions in the genome that co-segregate with a particular disorder within families, by comparing the greater frequency of markers in affected versus unaffected individuals, than would be expected by chance. Such identified markers are suggestive of chromosomal regions associated with the disorder, as it is assumed that the marker lies in close proximity to susceptibility genetic loci. ADHD molecular genetic research is characterised by a scarcity of linkage studies, driven by ADHD aetiology likely involving multiple genes of minor effect, which association approaches are more appropriate to identify (Faraone et al., 2005). The paucity of linkage studies emphasise the utility of combining studies for meta-analyses to increase power to detect reliable regions of interest (Zhou, Dempfle et al., 2008).

A meta-analysis pooling together seven independent genome-wide linkage studies (Zhou, Dempfle et al., 2008) found ten chromosomal regions suggestive of an association, but only one region (chromosome 16q) reached genome-wide levels of significance. Of particular interest, this region harbours the cadherin 13 (CDH13) gene which has emerged as an overlapping finding in genome-wide association studies (GWAS) (see section 1.4.1.4).

1.4.1.2 Candidate gene association studies

Association-based molecular genetic approaches compare frequencies of marker alleles in affected versus unaffected individuals, assuming that cases will exhibit higher frequencies of risk alleles than controls. Driven by pharmacological studies and knockout gene studies in mice,

the majority of association studies have focused on genes within the dopaminergic, norepinephrinergic and serotonergic transmitter systems (Waldman & Gizer, 2006).

The most recent comprehensive meta-analysis (Gizer et al., 2009) of ADHD candidate association studies, reported significant ($p < 0.05$) associations spanning six genes: the dopamine transporter (DAT1) gene; the dopamine D4 receptor (DRD4) gene; the dopamine D5 receptor (DRD5) gene; the serotonin transporter gene (SLC6A4, 5HTT); the serotonin 1B receptor (HTR1B, 5HT1B) gene; and the synaptosomal-associated protein 25 (SNAP-25) gene (see Table 1.2). However odd ratios ranged from 1.11 to 1.33, so markers confer a small increased risk. In addition, as none of the reported meta-analytic findings reached genome-wide significance tests (set at 5×10^{-8}), we cannot be fully confident that these findings reflect true associations (Asherson & Gurling, 2012). Significant heterogeneity in effect sizes across individual studies were found for some of these makers, leading the authors to conclude that future studies should identify sources of heterogeneity, as this will permit future studies to potentially maximise associations (Gizer et al., 2009).

1.4.1.3 Quantitative trait loci (QTL) approach

The majority of candidate gene association studies have targeted the genetic underpinnings of diagnosed ADHD. However, twin studies provide strong evidence supporting the conceptualisation of ADHD lying at the tail end of a continuous dimension (see section 1.3.2). Therefore targeting quantitative measures of ADHD behaviours, in line with the quantitative trait loci (QTL) approach, may be a valid alternative to examining the molecular genetic correlates of clinically derived categories (Zhou, Asherson et al., 2008). Rather than discovering novel ADHD susceptibility loci, the majority of existing QTL studies have investigated whether putative ADHD risk markers show similar associations with quantitative measures, thereby testing the feasibility of using a QTL approach (see chapter 4).

Table 1.2 Significant ($p < 0.05$) meta-analytic results for associations between candidate gene polymorphisms and childhood ADHD

Gene	Location	Polymorphism	Risk allele	Odd Ratios (95% CIs)	P value
DAT1	3'UTR	VNTR	10-repeat	1.12 (1.00 – 1.27)	0.03
DAT1	Intron 8	VNTR	3-repeat	1.25 (0.98 – 1.58)	0.03
DAT1	3'UTR	rs27072	'G' allele	1.20 (1.04-1.38)	0.006
DRD4	Exon 3	VNTR	7-repeat	1.33 (1.15 – 1.54)	0.00007
DRD4	Promoter	rs1800955	'T' allele	1.21 (1.04 – 1.41)	0.007
DRD5	5'Flank	Dinucleotide	148-bp allele	1.23 (1.06 – 1.43)	0.003
5HTT	Promoter	5HTTLPR	Long allele	1.17 (1.02- 1.20)	0.01
HTR1B	Exon 1	rs6296	'G' allele	1.11 (1.02 – 1.20)	0.01
SNAP-25	3'UTR	rs3746544	Unknown	1.15 (1.01- 1.31)	0.03

Note: Adapted from (Gizer et al., 2009); Risk alleles were included in analysis if they had been reported in at least four independent studies of clinically diagnosed DSM-IV ADHD; Abbreviations- DAT1: Dopamine transporter; DRD4: Dopamine D4 receptor; DRD5: Dopamine D5 receptor; 5HTT: Serotonin transporter; HTR1B: Serotonin receptor 1B; SNAP-25: Synaptosomal-associated protein 25; CIs: confidence intervals;

Overall the evidence is mixed regarding replicating putative ADHD risk markers with continuous measures of ADHD traits, in line with the heterogeneous findings reported for clinically derived categories. Pooling individual QTL studies may result in the identification of some more consistently replicated ADHD risk markers, such as found for candidate gene association studies on ADHD as a clinical disorder, but are currently lacking. Accordingly, we report findings from individual studies, but limit discussion to the polymorphisms outlined in Table 1.2. Moreover, this selective overview is limited to findings based on quantitative assessments of ADHD in terms of behavioural ratings, rather than the molecular genetic correlates of other ADHD-related traits. (See section 1.6.4.2 for molecular genetic correlates of ADHD-related cognitive impairments).

One study based on a subsample selected for high and low parental ADHD ratings from a population-based sample, found support for SLC6A4 as an ADHD risk gene (Curran, Purcell, Craig, Asherson, & Sham, 2005). Using the same sample, a significant association was observed between the 7-repeat allele of the DRD4 gene and high scoring individuals (Curran et al., 2001). In contrast, another study utilising multiple ADHD measures (parent and teacher ratings and interviews) across a number of time points (age seven, nine, 11, 13, 15, 18 and 26), found no associations with the same risk allele (Mill et al., 2002). Another study reported associations between the DRD4 7-repeat allele and both symptom domains, whereas associations with the 10-repeat allele of DAT1 were limited to inattentive symptoms (Bidwell et al., 2011). Nominal associations with DAT1 have also been reported for parental ADHD symptom ratings in a sample of two-year-old twins (Ilott, Saudino, Wood, & Asherson, 2010). A study targeting a composite score derived from ADHD parental ratings at age two, three, four, and seven in male DZ pairs, found no associations with putative ADHD risk alleles in DRD4, DRD5, and 5HT1B, but did find evidence to suggest that risk markers in DAT1 and SNAP-25 were QTL for ADHD ratings (Mill et al., 2005).

1.4.1.4 Genome-wide association studies (GWAS)

In contrast to candidate gene association studies, GWAS are hypothesis free and can scan the entire genome for novel genetic variants. However, in order to control for false positive errors due to multiple testing, genome-wide significance levels are set at 5×10^{-8} .

A meta-analysis of GWAS from four independent samples revealed no genome-wide significant associations, which the authors attributed to insufficient sample size and/or that genetic variance in ADHD is accounted for by extremely rare genetic variants (Neale et al., 2010). However, chromosome 7 was identified as a region of particular interest, harbouring eight single-nucleotide polymorphisms (SNPs) within the top 50 association results (Neale et al., 2010). In addition, the CDH13 gene ranked amongst the top hits from two independent GWAS (Lasky-Su, Neale et al., 2008; Lesch et al., 2008), and is found under the significant linkage peak identified in a meta-analysis (Zhou, Dempfle et al., 2008).

1.4.1.5 Summary of molecular genetic progress and future directions

In sum, the progress in identifying ADHD susceptibility genetic loci has not been as encouraging as initially hoped for. Candidate gene association studies are particularly hampered by non-replication. Factors contributing to the challenges involved include genetic complexity, genetic and clinical heterogeneity, and the non-optimal phenotypic definition of ADHD and a corresponding over-reliance on non-objective measures. In addition, heterogeneous findings may be a result of the interplay of environmental factors. Moreover, existing ADHD molecular genetic investigations have generally been underpowered to detect genes of minor effect, hypothesised to underlie ADHD. Accordingly, collaborative efforts are underway to combine samples and generate a large enough sample to detect such small genetic effects. This may be particularly fruitful for GWAS, which so far have yet to yield a genome-wide significant association.

In addition, there is increasing evidence that rare genetic variants (copy number variants (CNVs) (genetic variation on long sections of DNA, involving chromosomal duplications and deletions)) may play a significant role in ADHD, and other neurodevelopmental disorders such as ASDs (Elia et al., 2010; Williams, Zaharieva et al., 2010)). Further work is needed to resolve the extent to which CNVs associated with ADHD are inherited or arise *de novo* (Asherson & Gurling, 2012). Another interesting direction of research is generating polygenic risk indices that predict ADHD, although this has been hampered by the need for larger samples (Merwood & Asherson, 2011).

1.4.2 Environmental factors

Although beyond the scope of this overview it is important to note that in addition to a strong genetic component in ADHD, twin studies have highlighted the crucial role of environmental factors (Cortese, Faraone, & Sergeant, 2011; Faraone & Biederman, 2000). However, compared to the extensive research directed at discovering ADHD genetic risk markers, less focus has been subjected to the detection of specific environmental risk factors (Buschgens et al., 2008). This is despite the preventative implications of identifying causal links with environmental factors (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005).

The majority of existing research on environmental risk factors has focused on factors early in development, in line with ADHD being viewed as a neurodevelopmental condition (Nigg, 2006). A recent review concluded that environmental risk factors associated with ADHD traits include low birth weight, prematurity and early adversity (Thapar Cooper, Jefferies, & Stergiakouli, 2012). However determining causal links are problematic (Thapar et al., 2012; Thapar & Rutter, 2009), as teasing apart causal from inherited effects is challenging (Langley, Rice, van den Bree, & Thapar, 2005; Maughan, 2009).

1.4.3 GxE interactions

Some of the heterogeneity observed in candidate gene association studies (and studies on environmental risk factors) may stem from GxE interactive effects (Stevens et al., 2009). GxE interactive effects refer to the variation of genetic effects on disorder-risk based on environmental exposure, or the modification of environmental risk by different individual genotypes. Genetic factors contribute to approximately three-quarters of ADHD variance, which include GxE interactive effects (Kuntsi, Neale et al., 2006; Nigg, Nikolas, & Burt, 2010). Therefore, GxE investigations may contribute to accounting for a larger proportion of this inherited component.

Initial findings from GxE interaction studies are highly promising as interactive effects have emerged, even in cases where genetic main effects were non-significant (Kuntsi, Neale et al., 2006; Nigg, Nikolas et al., 2010). This underlies the important contribution of incorporating environmental risk factors into future genetic studies; even when susceptibility genes are

identified, environmental factors should also be considered as they may further moderate genetic effects (Thapar, Holmes, Poulton, & Harrington, 1999).

1.4.4 Summary of ADHD aetiological risk factors

ADHD is a complex multi-factorial disorder, likely the result of additive and interactive effects of both genetic and environmental factors. ADHD ranks amongst one of the most heritable childhood psychopathologies, but despite concerted efforts to detect genetic susceptibility loci, much of the genetic variance remains elusive (Kuntsi, Neale et al., 2006; Plomp et al., 2009; Purper-Ouakil et al., 2011). In addition, ADHD genetic association findings that retain significance in meta-analyses (although below genome-wide significance) suggest that they confer only a small increased risk. Moreover, the genetic complexity of ADHD and non-optimal phenotypic definition likely contribute to the challenges involved in identifying genetic ADHD risk markers. One way to overcome these obstacles may be to use endophenotypes as targets for molecular genetic studies or as a means of subdividing samples into more homogenous entities, although their main appeal is in bridging the gap between aetiological factors and clinical phenotypes (Meyer-Lindenberg, 2010) (see section 1.6).

1.5 Cognitive theoretical models of ADHD

The strong genetic component of ADHD is well established. However, this relates to the originating causes, rather than the underlying processes involved in the manifestation of ADHD (Nigg, 2006). In the following section we review key neurocognitive theoretical models of ADHD, based on psychological processes that may represent pathways mediating the genetic effects on ADHD symptoms.

1.5.1 Executive dysfunction theory of ADHD

Individuals with ADHD exhibit a range of deficits within executive functioning (EF). Although there is no universal consensus regarding the definition of EF (McQuade et al., 2011), it is an umbrella term reflective of higher order neurocognitive processes involved in attaining goal-directed behaviour. Key components include working memory, planning, inhibitory control,

cognitive flexibility and interference control (Pennington & Ozonoff, 1996), and a number of laboratory tasks have been developed to hone in on component operations (Nigg, 2005).

One of the earliest and initially prominent theories of ADHD, the executive dysfunction theory, hypothesised that dysfunctioning response inhibition was a primary deficit in ADHD, contributing to secondary EF impairments and leading to ADHD symptom presentation (Barkley, 1997). The model attributes ADHD behaviours as arising from deficient inhibitory control which impairs the ability of affected individuals to engage other executive-control mechanisms to regulate their behaviour.

ADHD is associated with significant impairments across several EF domains, as confirmed by a large meta-analysis of 83 studies (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). Documented impairments were not attributable to IQ or symptoms of co-occurring disorders (Willcutt et al., 2005). The strongest and most replicated deficits were observed for response inhibition, sustained attention, and spatial working memory (Willcutt et al., 2005). Overall, mean effect sizes were moderate, ranging from 0.43 to 0.69, with a weighted mean effect size of 0.54 across a total of 168 comparisons (Willcutt et al., 2005). The moderate, rather than high effect sizes, suggest substantial heterogeneity within ADHD, contributing to the authors' conclusion that such impairments are neither necessary nor sufficient causes for ADHD presentation (Willcutt et al., 2005). In addition, whilst no significant differences were found between ADHD-C and ADHD-PI subtypes, there was evidence (but based on only three studies) that executive impairments are less severe in individuals with ADHD-PHI subtype (Willcutt et al., 2005).

In a study across three sites comprising 887 participants, case-control group differences for virtually all EF measures were significant with medium effect sizes ranging from 0.35 to 0.91 (Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005). Despite these unequivocal group differences, using a cut-off point for EF impairment (90th percentile), no EF impairment was observed in more than 50% of children with ADHD-C subtype (Nigg et al., 2005), underlining the non-universality of EF deficits in ADHD. Although it is important to note that this (or any) cut-off is completely arbitrary, and so the distinction of the proportion of children who do or do not show impairments may not be so meaningful (Johnson, Wiersema, & Kuntsi, 2009).

Although there is strong evidence for EF deficits in ADHD, the effect sizes are moderate and deficits are not observed across all individuals with ADHD. In addition, as there is usually a publication bias against studies reporting negative findings, the effect sizes yielded from meta-analyses may overestimate the true extent of deficits (Halperin & Schulz, 2006).

1.5.2 Arousal dysfunction theories of ADHD

1.5.2.1 *The state-regulatory model*

The state-regulatory model (van der Meer, 2002) hypothesises a state-regulation deficit involved in ADHD, relating impaired arousal levels and effort-related resources contributing to difficulties in maintaining an optimal energetic state (particularly in unstimulating environments). The dysfunction in state-regulation is hypothesised as a key deficit in ADHD, underpinning observed cognitive impairments (Sergeant, 2005). The central tenant of the model is that the basic processes are largely intact in ADHD, but impaired state-regulation contributes to deficits in the allocation of cognitive resources. In other words: *“the engine is intact...but there is a problem with the petrol supply”* (van der Meer, 2002, p.189).

A key cognitive impairment associated with ADHD is increased reaction time variability (RTV, estimated as the standard deviation of reaction time (RT)), considered to reflect lapses of attention. It is hypothesised that the more variable (inconsistent) RT performance associated with ADHD, stems from sub-optimal arousal/effort levels. Accordingly, some studies have observed improved performance when task conditions are manipulated to optimise arousal/effort states. In a population-based twin sample, a faster rate of presentation and the use of incentives, normalised mean RT (MRT) and RTV in the go-no/go (GNG) and fast task (a four-choice RT task) in children with high ADHD symptom scores (Kuntsi, Wood, Van Der Meere, & Asherson, 2009). In contrast, the percentage of commission errors (CE, false alarms indexing inhibition deficits) on the GNG task did not improve with event rate manipulation or the use of incentives (Kuntsi et al., 2009).

Analysis on a large international collaborative sample of clinical probands with ADHD-C subtype and controls using the same tasks yielded a similar pattern of findings. The use of rewards and a faster presentation rate in the fast task, and incentives in the GNG task, led to improvements

in MRT and RTV across cases and controls, with the greatest effect on performance observed for ADHD probands (Andreou et al., 2007; Uebel et al., 2010).

The observation that RT performance can be moderated by event rate presentation and incentives suggests the involvement of energetic (arousal) and/or motivational (effortful) factors. The observed improvement according to task manipulation may stem from faster presentation rate increasing arousal levels and rewards contributing to increased effort. Joint analysis on the previously mentioned population-based twin sample and international collaborative sample of clinical ADHD proband and control sib-pairs investigated RTV difference scores from baseline to manipulated conditions, as a direct index of state-regulation (Kuntsi et al., in press). The findings indicated high phenotypic correlations and familial/genetic overlap between RTV difference scores in the GNG slow to either manipulated conditions (fast event rate or reward-related), suggesting that both largely index the same underlying process (Kuntsi et al., in press). In addition, the study reported high phenotypic and familial/genetic correlations between RTV difference scores and baseline RTV, underlining that both measures tap the same mechanisms (Kuntsi et al., in press). In light of these findings, the authors suggested the use of baseline RTV scores to overcome statistical issues related to difference scores.

1.5.2.2 The arousal-attention model

Another theoretical model of ADHD linked to impairments in modulating arousal levels is the arousal-attention model (Johnson, Kelly et al., 2007; O'Connell et al., 2008). However, in contrast to the state-regulatory model that attributes all impairments to arousal dysregulation, the arousal-attention model hypothesises that deficits in arousal regulation can only account for some observed impairments. Accordingly in the arousal-attention model, two separate processes are distinguished: bottom-up subcortical arousal regulation and top-down cortical control of sustained attention. Cognitive impairments are hypothesised to arise from deficits in these two components, with the first factor contributing to a decrement in vigilance according to declining arousal levels, and the second factor contributing to brief fluctuations in attention (Johnson, Kelly et al., 2007).

Analysis on the previously mentioned international collaborative sample of ADHD-C probands, siblings, and controls, revealed two distinct familial cognitive factors contributing to ADHD (Kuntsi et al., 2010). The larger factor, accounting for 85% of the familial variance of ADHD, accounted for nearly all the familial influences of RTV and MRT. This factor separated from a second familial factor that captured 62-82% of the familial influences on omission errors (OE, reflecting deficits in sustained attention) and CE, and accounted for 13% of the familial variance of ADHD (Kuntsi et al., 2010). In line with the attention-arousal ADHD model, the RT factor may reflect decrements in vigilance due to the drop in arousal levels, and the accuracy factor may reflect fluctuations of top-down control of sustained attention. The two-factor model is also consistent with the neurodevelopmental model (see section 1.5.3).

Overall there is substantial evidence for theories that emphasise altered arousal-regulation processes in ADHD. However, according to the state-regulatory model, inhibitory deficits in ADHD can be accounted for by inactivation of the inhibitory mechanism, rather than an actual inhibitory deficit. Yet, the evidence for improvements in inhibition, indexed by CE, is less consistent than that for RTV (Kuntsi et al., 2009). Therefore, the state-regulatory theoretical model can account for only some of the impairments associated with ADHD. In comparison to the evidence in favour of deficits in the modulation of arousal underlying ADHD impairments, fewer studies have tested the distinction outlined in the arousal-attention model, although the previously outlined two-factor (RT- and accuracy-related) model is consistent with the proposals of the arousal-attention model.

1.5.3 The neurodevelopmental model of ADHD

The neurodevelopmental model of ADHD (Halperin & Schulz, 2006) hypothesises that ADHD *aetiology* is linked to dysfunction in sub-cortical structures; whereas the *developmental trajectory* of ADHD (i.e. its persistence or remittance) is associated with maturation of the prefrontal cortex. Based on this distinction, aspects of cognitive performance related to more automatic processes (such as MRT and RTV), likely mediated by sub-cortical processing, will persist throughout development regardless of ADHD symptom manifestation (Halperin & Schulz, 2006). In contrast, deficits related to frontal-mediated effortful executive control (such as inhibition) will vary according to symptom presentation, remaining impaired only in those

with persistent ADHD (Halperin & Schulz, 2006). Thus, improvements in frontal-mediated cognitive functioning that parallel ADHD symptom attenuation are hypothesised to stem, in part, due to compensatory cognitive and neural mechanisms associated with prefrontal cortex maturation (Vaughn et al., 2011).

In line with the developmental model, using the continuous performance test (CPT) individuals with persistent and remittent ADHD showed increased RTV relative to controls, but only persisters differed from controls in relation to CE and OE (Halperin, Trampush, Miller, Marks, & Newcorn, 2008). In contrast, two other studies have found that CPT CE could not differentiate between those with persistent versus remittent ADHD, with CE impaired in both groups (Hinshaw, Owens, Sami, & Fargeon, 2006; Vaughn et al., 2011). However, these mixed findings may stem from differences in the time between initial and follow-up assessment. In the study that did find a difference the interval period was 10-years (Halperin et al., 2008), whereas the other two studies had shorter periods between reassessment (one year (Vaughn et al., 2011) and five years (Hinshaw et al., 2006)). Therefore, it may be the case that the longer follow-up interval captured the full difference in prefrontal maturation, considered to be one of the last regions of the brain to fully develop (Halperin et al., 2008). Accordingly, studies with a shorter period may miss the complete change in the prefrontal cortex.

The previously outlined two-factor model (distinguishing between RT- and accuracy- factors), is in line with the neurodevelopmental model (Kuntsi et al., 2010): with the RT factor potentially representative of stable (rather than transient) enduring deficits, and the accuracy-related factor potentially reflective of prefrontal-mediated executive control dysfunction.

1.5.4 Summary of cognitive theoretical models of ADHD

The overview of cognitive theoretical models of ADHD highlights RT- and accuracy- related factors among the key cognitive impairments in ADHD. This is supported by findings yielded on one of the largest samples to date, which reported the strongest phenotypic correlations with ADHD were for RTV and MRT (0.39 and 0.36, respectively), and slightly lower associations with OE and CE (0.22 and 0.19, respectively) (Kuntsi et al., 2010).

It is important to note that there are further theoretical models of ADHD, but it is beyond the scope of this selective overview to review competing theories on the underlying deficits of ADHD (see reviews by Johnson et al., 2009; Kuntsi & Klein, 2011; Sonuga-Barke, 2002, 2003). The clinical heterogeneity of ADHD likely reflects multiple causal pathways, and an increasing trend in the formulation of theoretical models is to account for this diversity by postulating multiple underlying processes. Increasingly findings from multidisciplinary studies are being integrated into theoretical models to account for ADHD heterogeneity. In particular, the investigation of objectively measured intermediate phenotypes (also referred to as endophenotypes), may contribute to delineating underlying pathophysiological processes.

1.6 Endophenotypes

Endophenotypes are objective disorder-related markers, and commonly encompass neurochemical, physiological, endocrinological, neuroanatomical and cognitive processes (Gottesman & Gould, 2003; Viding & Blakemore, 2007). Although there is substantial disagreement regarding all criteria to qualify as a valid endophenotype, there is almost universal consensus regarding the following four steps: 1) be reliable; 2) demonstrate heritability (be genetically influenced); 3) correlate with clinical disorder or its behavioural symptoms; and 4) share common genetic influences with clinical phenotype or its behavioural symptoms (Wood & Neale, 2010).

Endophenotypes may be further distinguished as either a risk indicator (correlates with disorder) or an intermediate phenotype (lies along the causal pathway between genetic factors and clinical phenotype) (Kendler & Neale, 2010). In essence the difference between these two concepts is that the former represents pleiotropic genetic effects (some of the same genetic markers contribute to increased risk of both phenotypes), whereas the latter represents mediating effects of the same genetic markers (Kendler & Neale, 2010). Yet, it is difficult to experimentally clarify whether an endophenotype covaries with a disorder due to pleiotropy or mediation, and this issue has largely been neglected (Kendler & Neale, 2010). (See chapter 4 where we test if shared genetic effects between ADHD ratings and cognitive endophenotypes reflect pleiotropic or mediating genetic effects).

Endophenotypes that are hypothesised to lie along the pathway between genetic factors and clinical phenotype are a promising tool to delineate pathophysiological processes underlying disorder presentation. Increased understanding of the neurobiological underpinnings of ADHD may contribute to facilitating intervention strategies, particularly by extending the study into animal models (Glahn & Blangero, 2011).

Although elucidating processes underlying ADHD is the main attraction of intermediate phenotypes (Kuntsi, Neale et al., 2006; Meyer-Lindenberg, 2010), some commentators (e.g. Gottesman & Gould, 2003) have additionally postulated that endophenotypes may prove advantageous for the detection of disorder genetic susceptibility loci. This optimism stems from endophenotypes being quantifiable bias-free measures, and being readily measured in the general population and therefore affording analysis of larger samples with greater power, as they are not limited to clinically ascertained samples (Losh, Sullivan, Trembath, & Piven, 2008). In addition, endophenotypes are hypothesised to be less genetically complex than the disorder itself, thereby increasing statistical power to detect genetic underpinnings. Despite galvanised interest, others have stressed that the utility of endophenotypes as viable targets for molecular genetic research is not definitive (Kuntsi, Neale et al., 2006). Molecular genetic investigations that target endophenotypes and identify overlapping genetic variants with the disorder will confirm the utility of this approach (see chapter 4). However, endophenotypes may yet prove beneficial for sampling strategies for molecular genetic studies, by providing a means of sub-typing clinically heterogeneous samples into more homogenous subgroups (Losh et al., 2008; Viding & Blakemore, 2007), even across current diagnostic categories (Levy & Ebstein, 2009).

In order to elucidate underlying neurobiological processes contributing to ADHD, the consideration of multiple intermediate phenotypes is a beneficial approach, as there are likely to be multiple pathways to ADHD (see section 1.5.4). Also considering the clinical heterogeneity of ADHD, diversity of neuropsychological deficits, and the partially distinct genetic aetiology of inattention and hyperactivity-impulsivity (see section 1.3.9), it is meaningful to look for underlying processes involved in the behavioural dimensions of ADHD separately (see chapter 3 and 4).

As previously highlighted (see section 1.5.4), RT-related factors and inhibition are key cognitive impairments in ADHD. There is stronger evidence for an inhibitory deficit in ADHD involving withholding a pre-potent response (as captured by GNG tasks), in contrast to inhibition related to suppression of a conflicting secondary response (as captured by interference control tasks such as the Stroop Colour Word Task or the Flanker task) (Nigg, 2001). In this section the evidence is reviewed for RT measures, and pure inhibition rather than interference, using the previously outlined criteria, to assess their suitability as candidate ADHD endophenotypes.

Two of the main studies referred to are based on the previously mentioned (section 1.5) population-based twin sample (Study of Activity and Impulsivity Levels in children (SAIL (n = 1312))), and an international collaborative sample of ADHD-C subtype probands, their siblings, and control sib-pairs (n = 1265). A number of the papers referred to in the subsequent sections were conducted on subsets of these two samples or preliminary analysis on the initial set of participants. These two studies employed the same cognitive tasks: the GNG and fast task.

The GNG task is an inhibition task. On each trial of the GNG task (Borger & van der Meere, 2000; Kuntsi, Andreou et al., 2005; van der Meere, Stemerding, & Gunning, 1995) one of two possible stimuli appeared for 300 milliseconds in the middle of the computer screen. The child was instructed to respond only to the 'go' stimuli and to react as quickly as possible, but to maintain a high level of accuracy. The proportion of 'go' stimuli to 'no-go' stimuli was 4:1. The participants performed the task under three conditions (slow, fast and incentive), matched for length of time on task.

The fast task is a standard warned four-choice RT task (Leth-Steensen, Elbaz, & Douglas, 2000). A warning signal (four empty circles, arranged side by side) first appeared on the screen. At the end of the fore period (presentation interval for the warning signal), the circle designated as the target signal for that trial was filled (coloured) in. The child was asked to make a compatible choice by pressing the response key that directly corresponded in position to the location of the target stimulus. Speed and accuracy were emphasized equally. The baseline condition of the fast task (Andreou et al., 2007; Kuntsi, Andreou et al., 2005; Kuntsi, Rogers et al., 2006) had a fore period of eight seconds and consisted of 72 trials. A comparison condition with a fast event rate (one second) and incentives followed the baseline condition (Andreou et al., 2007).

The variables obtained from the fast task include RTV and MRT, reflecting the speed and variability of speed of responding. In addition to RTV and MRT, the variables obtained from the GNG task include CE and OE (measures of accuracy). Heightened RTV (standard deviation of RT) is interpreted as lapses of attention. High CE (false alarms) index inhibitory deficits and high OE (failure to detect the target stimulus) reflect deficits in attention. In the SAIL sample there was a low frequency of OE, and so not included in further analysis. However, it is important to note that this may stem from the task paradigm: the GNG task typically targets CE (the proportion of 'go' to 'no-go' stimuli makes a response to a 'go' stimulus likely, whereas suppressing a response to 'no-go' stimuli is unlikely) (Kuntsi & Klein, 2011).

These two samples, tasks, and derived variables are the basis of some of the studies in this thesis (chapters 3 to 5). Therefore, these cognitive measures are now evaluated according to the endophenotype criteria outlined previously (see section 1.6).

1.6.1 Criteria 1: Endophenotype must be reliable

Analysis on a population-based sample of 49 children that were assessed on the GNG and fast task twice, with an interval of two weeks between testing sessions, revealed moderate to high test-retest reliability coefficients (0.47 to 0.85) for the majority of individual MRT, RTV, and CE across conditions (Kuntsi, Andreou, Ma, Borger, & van der Meere, 2005). Composites based on combining the baseline condition of the fast task and the slow condition of the GNG task led to increased test-retest reliability coefficients for MRT and RTV (Kuntsi et al., 2006).

In sum, CE derived from the GNG task, and MRT and RTV derived from the fast and GNG tasks, display good psychometric properties with substantial test-retest reliability for individual conditions, and even higher reliability for aggregate measures.

1.6.2 Criteria 2: Endophenotype should demonstrate heritability

Preliminary univariate analysis on the first 400 participants of SAIL (approximately 60% of the total sample), revealed that the highest heritability estimates for MRT (60%) and RTV (48%) were obtained when combining data across the baseline condition of the fast task and slow condition of the GNG task (Kuntsi, Rogers et al., 2006). Test-retest unreliability typically limits

heritability estimates, as measure error variance is subsumed by the non-shared environmental component. Therefore after correcting for test-retest reliability heritability estimates were higher, estimated at 73% and 68% respectively for MRT and RTV combined across fast task baseline and GNG slow condition (Kuntsi, Rogers et al., 2006). After correcting for test-retest reliability, heritability estimates for CE ranged from 32% to 67% across conditions of the GNG task (Kuntsi, Rogers et al., 2006).

In sum, moderate to high heritability have been reported for MRT, RTV and CE. In addition, heritability estimates are higher when aggregating across tasks and correcting for re-test reliability, factors that should be taken into account when refining the selection of which measures to take forward for molecular genetic studies.

1.6.3 Criteria 3: Endophenotype should correlate with clinical disorder/behavioural symptoms

Analysis on the ADHD and control sibling-pair sample revealed that compared to controls, cases displayed significantly slower MRT, heightened RTV, and increased CE and OE (Kuntsi et al., 2010). Phenotypic correlations with cognitive composite scores and ADHD were highest for RTV (0.39) and MRT (0.36); and lower for OE (0.22) and CE (0.19) (Kuntsi et al., 2010).

An examination of accuracy-related parameters (OE and CE), central tendency of RT (mean and median), and measures of dispersion (RTV (estimated as standard deviation of RT); coefficient of variation (CV) (estimated as RTV/MRT), which effectively controls for MRT; and consecutive variance (CON) (estimated as $\sqrt{(\sum(RT_i - RT_{i+1})^2)/(n-1)}$; (i =trial number, n =number of trials), which quantifies the amount of moment-to-moment fluctuations)), across a range of tasks in an independent sample of 110 children found significant impairments in the ADHD group (Klein, Wendling, Huettner, Ruder, & Peper, 2006). After controlling for IQ, compared to typically developing controls the ADHD group displayed significant impairments: more OE across the CPT; more CE across the CPT, and two N-back tasks; slower MRT for the CPT, GNG, and N-back tasks; and greater measures of dispersion (all three indices) across all tasks (Klein et al., 2006). The largest group differences were reported for dispersion-related measures, although this varied across tasks (Klein et al., 2006). Dispersion measures were also the best at discriminating groups, but again showed variation across tasks (Klein et al., 2006). After controlling for age, IQ,

median RT and errors, RTV was still able to significantly discriminate groups across all tasks (with the exception of the CPT) (Klein et al., 2006).

Despite variations in group differences according to task, a principle component analysis of dispersion measures revealed a one-factor solution for the ADHD group, suggesting that the association with ADHD is not dependent on task or specific dispersion measure, and can therefore be viewed as a unitary construct (Klein et al., 2006). This has important implications, such as allowing the aggregation of studies of dispersion-related measures across samples and across tasks, to provide greater statistical power for subsequent molecular genetic analyses (Wood et al., 2011).

Taken altogether, these findings suggest an association between ADHD and RTV, MRT, CE and OE, although the magnitude of the association can vary according to task selection. Measures related to accuracy (OE and CE) have a slightly weaker association than RT-related measures. Overall, the evidence is strongest and most consistent for RTV, ranking amongst one of the best cognitive deficits to discriminate groups according to ADHD affection status. In line with this, a review referred to increased RTV as “the one ubiquitous finding in ADHD research across a variety of speeded-reaction tasks, laboratories and cultures” (Castellanos & Tannock, 2002, p. 624).

It is important to note that the above studies could not look at differences according to ADHD subtype, either due to sample composition consisting of only ADHD-C subtype (Kuntsi et al., 2010), or too small a sample to further stratify by subtype (Klein et al., 2006). One study comparing performance on the GNG task between ADHD-PI and ADHD-C subtypes, found no significant differences in CE, OE, or RTV (Bidwell, Willcutt, Defries, & Pennington, 2007). However, there is a paucity of studies examining cognitive impairments in ADHD-PHI subtype (Nigg, 2005). This is an important direction for future research (Nigg, 2005; Williams, Hermens et al., 2010), as cognitive processes underlying ADHD are likely to differ according to ADHD subtypes.

Dimensional analysis on participants in the SAIL sample with both parent and teacher rated DSM-IV based ADHD symptoms (n = 1156 children (82% of sample)), revealed moderate

(around 0.20) correlations between RT-related measures and ADHD symptom scores (Kuntsi et al., 2009). Correlations for CE were significant, but lower (0.08 to 0.15) (Kuntsi et al., 2009). In line with the lack of research on subtype differentiation according to cognitive impairments, there is a paucity of population-based studies examining if cognitive deficits show differential covariation with inattentive versus hyperactivity-impulsivity symptoms (see chapter 3 where this is examined in the SAIL sample).

1.6.4 Criteria 4: Endophenotypes should share common genetic influences with clinical disorder/behavioural symptoms

1.6.4.1 Evidence for shared common genetic influences: quantitative genetic studies

Evaluation of this criterion has usually been conducted by measuring endophenotypes in probands, unaffected relatives of probands, and controls, and comparing group mean scores (Wood & Neale, 2010). If endophenotypes share genetic effects with the disorder, unaffected relatives of probands should show group mean scores intermediate of probands and controls, based on the fact that unaffected relatives of probands will share a portion of disorder-related risk genes (Bidwell et al., 2007). Although this approach can identify whether an endophenotype co-segregates with the disorder within families of probands, it cannot explicitly quantify the degree of familial sharing, which can be derived from model fitting (Wood & Neale, 2010).

Analysis on the international collaborative sample revealed moderate to high familial correlations (the degree of common familial (genetic and shared (C) environmental influences)) between ADHD and cognitive composites (RTV: 0.74, MRT: 0.61, CE: 0.45, and OE: 0.48), likely indexing shared genetic effects (Kuntsi et al., 2010). Familial correlations were particularly high between MRT and RTV (0.91) (Kuntsi et al., 2010). Subsequent analysis revealed that the association between ADHD and these cognitive impairments were largely independent (80% to 87%) of aetiological influences shared with IQ (Wood et al., 2010).

Twin studies are able to confirm whether the familial influences accounting for the covariation between an endophenotype and ADHD largely index shared genetic as opposed to shared environmental effects. In the SAIL sample genetic correlations (indicating the degree of

overlapping genetic effects) were high between ADHD behaviours and latent MRT (0.70) and RTV (0.74) factors (Wood, Asherson, van der Meere, & Kuntsi, 2010). Moreover, the shared aetiology between RTV and ADHD symptoms were largely independent (94%) of aetiological influences shared with IQ (Wood, Asherson et al., 2010).

Taken altogether, the findings suggest that RT measures have larger overlapping genetic influences with ADHD, than accuracy-related measures. Moreover, there are large shared genetic effects between MRT and RTV (as composites derived from GNG and fast task), suggesting that only one needs to be taken forward for molecular genetic association studies, where multiple testing is an issue. Furthermore, impaired general cognitive ability (as indexed by IQ) is unlikely to explain the specific deficits observed in ADHD. The consistency of these findings across clinical and population-based samples adds credence to the conceptualisation of ADHD as the extreme of a continuous dimension (Wood, Asherson et al., 2010) (see section 1.3.2).

1.6.4.2 Evidence for shared common genetic influences: molecular genetic studies

Meta-analyses implicate the 7-repeat allele of DRD4 in ADHD (Faraone, Doyle, Mick, & Biederman, 2001; Faraone et al., 2005; Gizer et al., 2009; Li, Sham, Owen, & He, 2006), which arguably ranks as one of the best replicated ADHD risk makers. The majority of studies have examined the molecular genetic correlates of RTV derived predominantly from CPTs (Kebir, Tabbane, Sengupta, & Joobar, 2009). Seemingly paradoxically, two reviews highlighted that the majority of candidate gene association studies report increased RTV (i.e. inferior cognitive performance) with the *absence* of this risk allele (Bellgrove, O'Connell, & Vance, 2008; Kebir et al., 2009). Furthermore, this finding seems specific to ADHD populations (Johnson et al., 2008). The interpretation of this unexpected finding, have included that the 7-repeat allele is associated with ADHD-related behavioural components rather than cognitive impairments (Swanson et al., 2000), or that both increased and decreased dopamine levels contribute to impaired RT performance (Doyle, Willcutt et al., 2005). Both reviews concluded that overall the evidence is most consistent for the absence of the 7-repeat allele to be associated with increased RTV, versus other cognitive deficits associated with ADHD (Bellgrove et al., 2008; Kebir et al., 2009).

The most recent meta-analyses yielded a positive association between ADHD and the 10-repeat allele of DAT1 3'UTR (Gizer et al., 2009). In line with the 10-repeat allele conferring ADHD risk, increased RTV, CE, and OE are reported to be associated with 10-repeat homozygosity, although this is not universally found (see (Bellgrove et al., 2008; Kebir et al., 2009) for more details).

In sum, there is relatively consistent evidence for an association with increased RTV and the absence of the 7-repeat allele of DRD4, and substantial support for an association between RTV and homozygosity of the 10-repeat DAT1 allele (Bellgrove et al., 2008; Kebir et al., 2009). The evidence regarding the genetic correlates of MRT, CE and OE is less consistent (see (Bellgrove et al., 2008; Kebir et al., 2009)). Unfortunately, the array of tasks and measures assessed are so varied that a meta-analysis of individual studies is currently lacking (Kebir et al., 2009), although this would go some way to identifying and quantifying more consistently replicated associations. Yet, the previously noted unitary construct observed for RTV (Klein et al., 2006) (see section 1.6.3), suggests that pooling together individual studies may be appropriate.

1.6.5 Summary

In sum, there is convincing evidence that the cognitive deficits explored above are worthwhile ADHD endophenotypes. The evidence is strongest for RTV across the entire criteria, although the two-factor model derived from analysis on the sample of ADHD-C subtype probands, siblings and control sib-pairs (see 1.5.2.2) suggests that RTV cannot fully account for ADHD, and that there is evidence for an additional underlying process involving accuracy-related factors. These cognitive factors are explored in more detail in this thesis. In line with findings that report the partially distinct aetiologies of inattention and hyperactivity-impulsivity (see section 1.3.9), we investigate phenotypic and aetiological covariation of these cognitive impairment factors with the separate ADHD symptom subscales (chapters 3-4). We further test whether the shared genetic effects, represent pleiotropic or mediating genetic effects (chapter 4).

In addition, we investigate the association of these cognitive impairment factors with the symptom subscales of autistic-like traits (ALTs) (see chapter 5). Twin studies report substantial overlapping genetic effects between ADHD behaviours and ALTs (see section 5.2), and a review

on the overlap between ADHD and ASDs highlighted the search for common endophenotypes as a crucial future direction in clarifying the link between these two disorders (Rommelse, Geurts, Franke, Buitelaar, & Hartman, 2011) (see chapter 5). The identification of endophenotypes that are shared across multiple disorders or disorder-specific can illuminate shared or unique gene-brain/cognitive-behavioural processes (Doyle, Faraone et al., 2005).

1.7 The link between ADHD and ASD

1.7.1 ASDs

ASDs are characterised by impairments in social interaction and communication and the presence of restricted repetitive behaviours and interests. ASDs can be conceptualised as a heterogeneous group of neurodevelopmental disorders varying along a continuum of severity (Freitag, Staal, Klauck, Duketis, & Waltes, 2010; Sheinkopf, 2005), and include Autism, Aspergers Syndrome and Pervasive Developmental Disorder not otherwise specified (PDD-NOS), subsumed under the DSM-IV category of Pervasive Developmental Disorders (PDDs) (Ronald & Hoekstra, 2011). Autism is the most severe ASD and frequently associated with learning difficulties and language delay, whereas children with Aspergers Syndrome generally display significant impairments in social interaction, but intact cognitive ability and no language delay. A diagnosis of PDD-NOS is given when a child does not meet full criteria for another PDD but still experiences functional impairments, or when all symptoms are present but symptom onset was after the age of three.

1.7.2 Clinical overlap between ADHD and ASD

A recent review of clinical samples suggested that 20-50% of children with ADHD also meet diagnostic criteria for an ASD, and 30-80% of children with ASD meet criteria for ADHD (Rommelse, Franke, Geurts, Hartman, & Buitelaar, 2010). ADHD was also the second most frequently diagnosed co-occurring disorder from structured parental interviews in a population-based sample of children diagnosed with ASD, with a three-month point prevalence rate of 28% (Simonoff et al., 2008). In a large Swedish nationally-representative study using information from parental telephone interviews (and so not equivalent to a clinical diagnosis assessment), 51% of children with an ASD also met criteria for ADHD, and 23% of children with

ADHD also met criteria for an ASD (Lichtenstein, Carlstrom, Rastam, Gillberg, & Anckarsater, 2010). Analysis of behavioural ratings in the TEDS sample at age eight identified 77 unrelated children who met criteria for an ASD, of which 41% also had suspected ADHD, and 22% of the 137 unrelated children who met criteria for ADHD also met criteria for an ASD (Ronald, Simonoff, Kuntsi, Asherson, & Plomin, 2008).

Taken together these results suggest that the risk of these two disorders co-occurring together is high. In accordance with the observed frequent co-occurrence of these two disorders, upcoming diagnostic criteria are likely to amend the current prohibition of a diagnosis of ADHD with the simultaneous presentation of a PDD (Coghill & Seth, 2011).

1.7.3 Familial overlap: autistic-like traits in families of ADHD probands

Two separate family-based studies from the International Multi-Centre ADHD Genetics (IMAGE) study have examined the familial co-occurrence of ALTs in children with ADHD-C subtype and their unaffected siblings compared to control sib-pairs (Mulligan et al., 2009; Nijmeijer et al., 2009). The larger study consisting of samples across multiple sites, included over 800 ADHD-C subtype probands and over 100 siblings aged five to 17 (Mulligan et al., 2009). This study reported a high phenotypic correlation between Social Communication Questionnaire (SCQ) ALT scores and DSM-IV based ADHD scores for males (0.63), with over half (56%) of the association due to familial factors (Mulligan et al., 2009). The authors hypothesised that the familial influences largely index genetic effects, based on consistent findings of a substantial common genetic aetiology for these two behaviours from twin studies (see section 5.2). In females a slightly lower, but still moderate, phenotypic correlation was observed between ADHD and ALT scores (0.49), but the results did not indicate that ALTs were familial with ADHD in females (Mulligan et al., 2009). However, the authors noted that the large gender discrepancy in the sample (88% of probands were male), potentially contributed to unreliable cross-correlations, and therefore conclusions regarding the familial overlap of ADHD and ALTs in females could not be reliably ascertained.

In addition, children with ADHD-C subtype that presented with low ALT scores had a lower prevalence of co-occurring disorders, versus children presenting with high ALT scores (Mulligan

et al., 2009). The latent class group with the highest mean ALT score had the highest prevalence of comorbid ODD, CD, and language- and motor- disorders (Mulligan et al., 2009). The authors hypothesised that this implicates that comorbid ADHD and ASD may represent a distinct subgroup of ADHD, characterised by high levels of co-occurring disorders.

The smaller study, consisted of 256 ADHD-C subtype probands-sibling pairs, and 147 controls aged between five and 19 (Nijmeijer et al., 2009). In this study ADHD behaviours were rated using DSM-IV based scales, and ALTs using the Children's Social Behavior Questionnaire (CSBQ). Phenotypic correlations between the CSBQ subscales representing core problem areas (social, understanding, change and stereotyped) were greater for inattention versus hyperactivity-impulsivity (Nijmeijer et al., 2009). Moreover, the largest differentiation for these subscales was found for the social ALT subscale, which correlated stronger with inattention ($r = 0.23$, $p < 0.001$) but was low and non-significant with hyperactivity-impulsivity ($r = 0.08$) (Nijmeijer et al., 2009).

In line with the previous study, probands and their siblings had higher ALT scores than controls; in contrast, sibling correlations were higher in female probands suggesting that ALTs may be more familial for females with ADHD (Nijmeijer et al., 2009). However, in contrast to the previous study, all cross-correlations between proband PDD subscale symptom scores and sibling ADHD subscale symptom scores were low and non-significant (-0.01 to 0.10), suggesting the familial independence of ADHD and PDD symptom (Nijmeijer et al., 2009).

The difference between the two studies could stem from the latter study having a much smaller sample size (Ronald, Edelson, Asherson, & Saudino, 2010). Another explanation may be the use of different assessment instruments to assess ALTs, as the scale employed in the latter study (CSBQ) adopts a three-point Likert-scale (in contrast to yes or no responses required in the SCQ) and also measures less severe ALTs (Rommelse et al., 2010).

1.7.4 Genetic overlap between ADHD and ASD

The overwhelming evidence from twin studies suggests that both ADHD behaviours and ALTs correlate moderately, and share a common genetic aetiology (see section 5.2). This finding is

consistent across genders, informants and assessment instruments, and evident from age two, although there is evidence of a developmental increase (Ronald, Edelson et al., 2010). Accordingly studies have attempted to identify common genes (see Rommelse et al., (2010) for a review of molecular genetic studies). However, a limitation of existing twin studies, is that they fail to take into account the great genetic heterogeneity observed within the symptom subscales of ALTs (Dworzynski, Happe, Bolton, & Ronald, 2009; Ronald et al., 2006; Ronald, Happe, & Plomin, 2005; Ronald, Happe, Price, Baron-Cohen, & Plomin, 2006), and the partially distinct aetiologies of ADHD behaviours (Greven, Rijdsdijk et al., 2011; McLoughlin et al., 2007). This is a significant gap in the literature as investigating symptom domains separately may clarify the link between these two disorders, facilitating the identification of pleiotropic genes and elucidating shared or unique gene-behaviour pathways (see chapter 5).

1.8 ADHD and atypical hypothalamic-pituitary-adrenal (HPA) axis activity

1.8.1 The HPA axis

The hypothalamic-pituitary-adrenal (HPA) axis is a system of direct interactive influences and negative feedback interactions involved in stress adaptation. Activation of the HPA axis on presentation of a stressor results in an increase in several hormones, with increased levels of cortisol as an end-product. As the HPA axis is involved in stress-regulating mechanism, cortisol can be considered as indexing arousal (Stadler et al., 2011; Wang et al., 2011). Cortisol is also produced throughout the day and follows a diurnal rhythm: characterised by high levels at awakening, peaking approximately 30 minutes post-awakening (referred to as the cortisol awakening response (CAR)), and declining throughout the rest of day (Alink et al., 2008).

1.8.2 The HPA axis and adverse health outcomes

Atypical HPA axis functioning have been associated with a range of adverse health outcomes. Specifically, hyperactivity of the HPA axis (elevated cortisol productivity) are associated with anxiety-related disorders (Greaves-Lord et al., 2007), major depressive disorders (Hinkelmann et al., 2009), and bipolar disorder (Daban, Vieta, Mackin, & Young, 2005). Blunted HPA axis activity (hypoactivity) has been associated with externalising behaviours, such as aggression

(McBurnett, Lahey, Rathouz, & Loeber, 2000), CD (Fairchild et al., 2008; Oosterlaan, Geurts, Knol, & Sergeant, 2005), and oppositional defiant behaviour (van Goozen et al., 1998).

A meta-analysis found a small but significant inverse association between externalising behaviours and baseline cortisol levels (Alink et al., 2008). This relationship was stronger in clinical samples or samples predominantly consisting of males. A further moderating factor was age: higher externalising behaviours were associated with higher baseline cortisol in pre-schoolers, and lower concentrations in school-aged children, and no significant association in adolescents (Alink et al., 2008). These variations highlight developmental changes in HPA axis functioning. In contrast, the meta-analysis found no association between externalising behaviours and cortisol reactivity, although the number of available studies was less than half the pooled number of studies investigating baseline cortisol (Alink et al., 2008). The authors hypothesised that the lack of a significant association with cortisol reactivity, and the small effect size observed with baseline cortisol, may be attributable to the behavioural heterogeneity encompassed by externalising behaviours. Moreover, many of the included studies did not take into account co-occurring disorders, which can also impact HPA activity (Alink et al., 2008).

1.8.3 The HPA axis and ADHD

Research on HPA axis functioning in ADHD has investigated both stress-induced cortisol concentrations and diurnal variation, with greater emphasis directed at stress reactivity. One of the earliest studies to observe atypical HPA axis functioning in ADHD found the association limited to children with persistent ADHD (at two-year follow-up) (King, Barkley, & Barrett, 1998). Only two studies have subsequently been conducted on adults with ADHD, which failed to replicate an association with persistent ADHD (Hirvikoski, Lindholm, Nordenstrom, Nordstrom, & Lajic, 2009; Lackschewitz, Huth, & Kroner-Herwig, 2008).

The association with altered regulation of the HPA axis (specifically blunted cortisol activity), is a relatively frequent finding from studies conducted on children with ADHD (see section 6.2). However, this finding is not ubiquitous and overall the research suggests that only a subset of individuals with ADHD display atypical HPA axis activity (Stadler et al., 2011; Yang, Shin, Noh, &

Stein, 2007). The possible heterogeneity in HPA functioning within ADHD may account for some of the inconsistent findings (Stadler et al., 2011; Yang et al., 2007). Accordingly, an increasing trend is to subdivide ADHD samples according to cortisol reactivity, to elucidate behavioural correlates of atypical HPA axis activity. Moreover, stratification by HPA axis activity can be used to create more homogenous subgroups, which may prove useful for molecular genetic investigations of ADHD potentially hampered by ADHD heterogeneity. A series of studies that subdivided clinical referred children with ADHD according to cortisol reactivity to a cognitive stressor (Lee, Shin, & Stein, 2010; Shin & Lee, 2007), identified lower IQ and CBCL AP scores as characteristic of ADHD individuals who displayed blunted cortisol reactivity (Shin & Lee, 2007).

Attenuated cortisol (re)activity may reflect under-arousal, and is therefore in line with arousal dysregulation models of ADHD previously outlined (see section 1.5.2). Only one study has examined the association of cortisol reactivity with RTV performance in individuals with ADHD (Lee et al., 2010). This study adopted a similar approach as outlined above, categorising children with ADHD as either responders or non-responders to a cognitive stressor according to cortisol reactivity (increase or decrease). ADHD participants who demonstrated an increase in cortisol levels displayed increased RTV (Lee et al., 2010). If cortisol is assumed to index arousal levels, this suggests that impaired RTV performance is not only linked to under-arousal (as hypothesised by the state-regulatory and arousal-attention models of ADHD (see section 1.5.2)), but that RTV can also be impaired when over-aroused. Accordingly deviations from an optimal state of arousal, either in terms of over- or under- arousal, may negatively impact RTV performance in individuals with ADHD. However, as this study did not include a control group the generalisability of this finding could not be ascertained.

The association between ADHD and atypical HPA is complicated by a number of potential moderators. Although an increasing number of studies are investigating HPA axis functioning in ADHD, a key issue remains whether the association previously observed is due to commonly co-occurring behaviours (Corominas et al., 2012) (investigated in chapter 6).

1.9 Interim summary

This chapter reviewed key findings in relation to the ADHD phenotype at a number of levels: the epidemiology and clinical profile, the genetic aetiology, and at the neurocognitive and physiological (as indexed by salivary cortisol) levels. This thesis adopts a multidisciplinary approach, combining quantitative and molecular genetic analysis, and using genotyping data, behavioural ratings, and cognitive-experimental and physiological measures, to investigate pathways from genes to ADHD behaviours, and to investigate the specificity of pathways and moderating effects of co-occurring behaviours.

1.10 Specific aims and objectives of thesis

Despite a strong genetic component, much of the genetic variance of ADHD remains unaccounted for (Kuntsi, Neale et al., 2006; Plomp et al., 2009; Purper-Ouakil et al., 2011). The non-optimal phenotypic definition of ADHD and an over-reliance on rating scales, likely contribute to the challenges involved. Moreover, the accuracy of the most common informant of childhood ADHD symptoms – parents – is compromised as a result of rater contrast effects (see section 1.3.7.2). However, with the exception of rating-scale characteristics, we know little about the factors that contribute to rater contrast effects in parental ADHD ratings. The improved identification of the factors that contribute to parental rater bias has important implications for future research on ADHD.

Based on this gap in the literature, and using a large population-based sample with multiple ADHD parental behavioural ratings across a number of time points, we aimed to:

- investigate if contrast effects in parental ADHD ratings differ according to gender composition of rated twins (chapter 1);
- estimate the interactive role of gender composition of twin pairs with other socio-demographic factors (ethnicity, socio-economic status (SES) and family size) that may contribute to parental rater contrast effects (chapter 1).

The subjective nature of rating scales has contributed to great interest in obtaining ADHD-related objective measures. In addition, such bias-free measures may facilitate the identification of complex pathways from genes to behaviour. The review of cognitive theoretical models of ADHD (see section 1.5) and of cognitive deficits examined in more detail in this thesis (see section 1.6), highlight the potential utility of RTV and CE as viable endophenotypes to investigate causal processes underlining ADHD. The investigation of causal pathways mediating ADHD is still in its infancy (Kuntsi & Klein, 2011), and it is beyond the scope of this thesis to review competing theories on the underlying deficits of ADHD. Rather the focus of this thesis is to investigate the specificity of neuropsychological pathways to ADHD behaviours, and whether cognitive impairment factors are useful targets for molecular genetic research.

Specifically, the objectives we seek to achieve in the second part of the thesis are the following:

- investigate the aetiological associations between cognitive impairments and the two symptom domains of ADHD considered separately (chapter 3);
- investigate if the aetiological separation between impaired RT performance and CE previously observed in a clinical sample is also observed in a population-based sample (chapter 3);
- investigate whether cognitive impairment factors and ADHD behavioural ratings show genetic associations with putative ADHD risk genetic markers (chapter 4);
- investigate whether overlapping genetic associations reflect pleiotropic or mediating genetic effects (chapter 4);
- investigate the aetiological association between ALTs decomposed into social and non-social subscales, and the two symptom domains of ADHD considered separately (chapter 5);
- investigate the aetiological specificity of RTV and CE to ADHD, by investigating the degree of phenotypic and aetiological associations of these cognitive impairments with social and non-social ALTs (chapter 5);

Increasing research has suggested that ADHD is associated with atypical HPA axis activity, although this association may be confounded by commonly co-occurring behaviours. Moreover, no study to date has examined the aetiological overlap between ADHD and indices of HPA axis activity. This study aimed to investigate the association between ADHD affection status and cortisol reactivity and diurnal variation, in a sample of male adolescent twin pairs selected from a population-based sample for high and low ADHD symptoms.

Specifically in this final empirical chapter we sought to:

- examine group differences in HPA axis activity, indexed by salivary cortisol (chapter 6);
- explore the phenotypic association and aetiological overlap between ADHD affection status and indices of cortisol activity, including those derived from growth curve modelling (chapter 6);
- explore the moderating effects of anxiety-shy symptoms and oppositional behaviours on the association between ADHD and growth curve parameters of cortisol activity (chapter 6).

CHAPTER 2 BIGGER FAMILIES FARE BETTER: A NOVEL METHOD TO ESTIMATE RATER CONTRAST EFFECTS IN PARENTAL RATINGS ON ADHD SYMPTOMS

2.1 Abstract

Many twin studies on parental ratings of attention-deficit/hyperactivity disorder (ADHD) symptoms report low or negative dizygotic (DZ) twin correlations. The observed differences in variances by zygosity indicate sibling contrast effects, which appear to reflect a bias in parent ratings. Overall, our knowledge of the factors that contribute to this rater contrast effect is, however, limited. Using parent-rated ADHD symptoms from the Twins' Early Development Study (TEDS) and a novel application of a twin model, we explored a range of socio-demographic variables (ethnicity, socio-economic status (SES), and family size) as potential contributors to contrast effects and their interactive effect with gender composition of twin pairs. Gender did moderate contrast effects but only in DZ opposite-sex twin pairs. Family size also showed a moderating effect on rater contrast effects, which was further modified by gender. We further observed an effect of rating scale, with the DSM-IV ADHD subscale of the Revised Conners' Parent Rating Scale more resistant to contrast effects than shorter rating scales of ADHD behaviours. The improved identification of situations where the accuracy of the most common informant of childhood ADHD symptoms – parents – is compromised as a result of rater bias, may have implications for future research on ADHD.

2.2 Introduction

Attention-deficit/hyperactivity disorder (ADHD) is characterised by developmentally inappropriate levels of hyperactivity, impulsivity and inattention, and is one of the most frequently diagnosed childhood-onset disorders. Pooled quantitative genetic studies yield mean heritability estimates of 0.70 to 0.76 (Biederman & Faraone, 2005; Burt, 2009), making ADHD one of the most heritable psychiatric disorders (Plomin et al., 2008). However, rater contrast effects in parental ratings artificially amplify differences between monozygotic (MZ) and dizygotic (DZ) twin correlations, yielding potentially inflated heritability estimates (Freitag, Rohde, Lempp, & Romanos, 2010; Wood, Buitelaar et al., 2010). In line with this, a review reported lower heritability estimates for teacher-rated ADHD symptoms (around 50%) and actigraph-measured estimates of activity level (30-52%) (Freitag, Rohde et al., 2010).

Several twin studies on parental ADHD ratings have reported low DZ correlations that are less than half of MZ twin correlations, indicating potential genetic dominance or rater contrast effects (Kuntsi et al., 2004; Price et al., 2005; Rietveld, Hudziak et al., 2003; Saudino et al., 2005). Low DZ correlations in tandem with significantly larger DZ variances (relative to MZ) indicate contrast effects, which may be attributed to either competitive sibling interaction whereby the behaviour of one twin influences the behaviour of the co-twin (reflecting true phenotypic differences), or a form of rater bias where parents emphasize behavioural differences. The evidence suggests parental rater bias (Simonoff et al., 1998) (see section 1.3.7.2), supported by the lack of low DZ correlations for objective measures of ADHD-related behaviours (Saudino et al., 2000; Saudino, Wertz, Gagne, & Chawla, 2004) and teacher ADHD ratings (Martin et al., 2002; Saudino et al., 2005). The absence of rater contrast effects in teacher ratings are hypothesised to stem from their greater exposure to children and behavioural norms (Hartman et al., 2007).

ADHD ratings seem particularly susceptible to parental rater contrast effects (Saudino et al., 2005) (see section 1.3.7.2), perhaps because they are based on more subjective criteria. Parental ratings of other behavioural traits, such as conduct problems, show no contrast effects (Hudziak, Derks, Althoff, Copeland, & Boomsma, 2005; Saudino et al., 2005), potentially attributable to increased awareness of clearly defined societal norms for more socially disruptive behaviours (Simonoff et al., 1998).

However, contrast effects are not universally found in parental ratings of ADHD, but vary according to a number of (potentially interacting) factors. A first factor to consider is the psychometric properties of the rating scale (Thapar et al., 2000). Overall, contrast effects seem less likely to arise when rating scales contain specific descriptions of behaviour (Saudino et al., 2004), are longer and more detailed (Kuntsi, Rijdsdijk et al., 2005) such as DSM-IV symptom checklists, and in scales with a broader scoring range (Hay et al., 2007; Polderman et al., 2007).

Another factor that might influence rater contrast effects of parental ADHD ratings is the age of the child being rated. A previous study based on the present sample (Twins' Early Development Study (TEDS)) reported rater bias in parental ADHD ratings in children aged two, three and four (Price et al., 2005). In line with this, a study based on a sub-sample of TEDS found contrast

effects in DSM-IV ADHD ratings at age five (Kuntsi et al., 2004). Yet, follow-up studies in TEDS extending to middle-childhood and early adolescence report no contrast effects in ratings obtained from the DSM-IV based Revised Conners' Parent Rating Scale at age eight (Kuntsi, Rijdsdijk et al., 2005; McLoughlin et al., 2007) or 12 (Greven, Rijdsdijk et al., 2011), suggestive of a developmental decline. Within the TEDS sample, comparisons of cross-sectional analyses on parental ratings from the Strengths and Difficulties Questionnaire for preschool twins (Price et al., 2005; Price et al., 2001) and twins aged 12 (Merwood et al., Submitted), also suggest a developmental decline in the magnitude of parental contrast effects. This pattern of results has also been found in an independent twin sample for maternal Child Behavior Checklist (CBCL) ADHD-related ratings, which the authors hypothesised could be attributable to parents increased exposure to children as their own children get older (Rietveld, Hudziak et al., 2003).

A third factor, gender, may also influence rater contrast effects of parental ADHD ratings. One study found evidence of contrast effects in parental ADHD ratings, which was limited to ratings of females (Vierikko et al., 2004). In contrast, another two studies on independent twin samples found that contrast effect parameters could be equated by across same-sex pairs (Rietveld, Hudziak et al., 2003; Simonoff et al., 1998), suggesting that at least within same-sex pairs, parents tendency to rate one twins ADHD-related behaviours in relation to their co-twin was not moderated by gender. Moreover, these two studies showed that contrast effects could be further constrained between same-sex and opposite-sex twin pairs, suggesting that even in cases when twin members are not of the same gender, they are compared to a similar extent as same-sex pairs (Rietveld, Hudziak et al., 2003; Simonoff et al., 1998). In contrast, another study reported significantly larger contrast effects for same-sex versus opposite-sex pairs, suggesting that when twin members of a pair differ by gender, ADHD-related ratings are made more independently (less influenced by rater contrast effects) (van Beijsterveldt et al., 2004). Only one of these studies has further decomposed contrast effect parameters within DZ opposite-sex pairs, estimating a contrast effect parameter from males-to-females (the female member is evaluated in relation to their male co-twin) and from females-to-males (the male member is evaluated in relation to their female co-twin) (Rietveld, Hudziak et al., 2003). In this study, rater contrast parameters did differ by gender within opposite-sex pairs, with a larger effect observed from males-to-females compared to from females-to-males, suggesting that when opposite-sex pairs are being rated for ADHD-related behaviours, the male twin is

considered the standard and the female twin evaluated accordingly (Rietveld, Hudziak et al., 2003). The authors hypothesised that the tendency to use males as a comparative benchmark may stem from ADHD-related behaviours being more commonly associated as male traits and more frequently observed in males (Rietveld, Hudziak et al., 2003).

The aim of this paper is to explore potential explanatory factors for contrast effects, employing a novel non-genetic model; therefore we do not investigate aetiological components of ADHD. Few studies have investigated additional socio-demographic factors that may influence contrast effects. One study reported no effect of parental socio-economic status (SES) or education, as contributing to contrasting non-twin siblings (Saudino et al., 2004). Although sibling-pair constellation variables, such as number of children, sex composition, and age distribution, have been hypothesised as contributing to the tendency to contrast children (Carey, 1986), behavioural difference scores (indexing contrast effects) were not correlated with differences in gender and age composition in non-twin siblings (Saudino et al., 2004). Family size or ethnicity, have not yet been formally investigated as moderating the process of contrasting siblings. The improved identification of situations where the accuracy of the most common informant of childhood ADHD symptoms – parents – is compromised, might contribute to our theoretical understanding of this puzzling effect. In this study we examine several parent- and child-related socio-demographic characteristics as potential factors that may contribute to contrast effects. Specifically, we present a model which will enable us to: (1) explore if contrast effects in parental ADHD ratings differ according to gender composition of rated twins; and (2) estimate the interactive role of gender with other demographic factors (ethnicity, SES, and family size) that may contribute to parental rater contrast effects, using repeated measures of the Revised Parent Rutter Scale for Pre-School Children (Hogg, Rutter, & Richman, 1997), Strengths and Difficulties Questionnaire (Goodman, 1997), and the Revised Conners' Parent Rating Scale (Conners, Sitarenios, Parker, & Epstein, 1998a), at a number of time points from early childhood to pre-adolescence.

2.3 Methodology

2.3.1 Sample

Participants are members of TEDS (Trouton, Spinath, & Plomin, 2002), a population-based birth

cohort of twins born in 1994-1996. All families in England and Wales identified by the Office for National Statistics as having twins born in these years were invited to enrol when the twins were aged 18 months old. Parents of all participants have provided informed consent and the study has been approved by the Institute of Psychiatry Ethical Committee (approval number 183/94). The 18-month booklet contained questions relating to pregnancy, birth, and socio-demographic indicators. Zygosity status was initially assigned based on a standard parent-rated zygosity questionnaire that has been shown to have a greater than 95% accuracy rate, compared to zygosity determined by DNA testing (Price et al., 2000). Zygosity for the vast majority of the sample has been subsequently confirmed by the employment of DNA markers. Despite attrition and non-responses over time, TEDS families at each age remain reasonably representative of the UK population in terms of parental education, parental employment and ethnicity (Oliver & Plomin, 2007; Trouton et al., 2002).

Twin pairs were excluded from the current analysis if there were extreme pregnancy or perinatal difficulties, specific medical syndromes and chromosomal anomalies, or if first contact data or zygosity information was unavailable. After exclusion criteria, symptom scores using the Revised Parent Rutter Scale for Pre-School Children (Hogg et al., 1997) at ages two, three and four, were obtained for 9153, 9437, 12974 individual twins, respectively. At ages four, seven, and 12, symptom scores were derived from the Strengths and Difficulties Questionnaire (Goodman, 1997) for 12966, 14359, 11170 individual twins. At ages eight and 12, ratings from the DSM-IV based ADHD subscales of the Long Version of the Revised Conners' Parent Rating Scale (Conners et al., 1998a) were available for 12518 and 11181 individual twins.

2.3.2 Measures

2.3.2.1 ADHD symptoms

At the two-, three-, and four-year data collection points, parents were asked to rate the behaviour of each twin using the Revised Parent Rutter Scale for Pre-School Children (RRPSPS) (Hogg et al., 1997). The rater reported on the frequency of behavioural attributes using a three-point scale: 0 indicates a response of 'not true', 1 indicates 'sometimes true' and 2 indicates 'certainly true'. The current analyses focused on four items that make up the hyperactivity-inattentive subscale ("restless; runs about or jumps up and down, doesn't keep still"; "squirmy,

“fidgety”; “has poor concentration, or short attention span”; and “inattentive”). The total ADHD symptom score was calculated by summing scores for each rated item, with a higher score inferring increased levels of ADHD-related behaviours. In a few cases, missing responses to individual items were pro-rated: a summary score based on the mean of remaining individual questions on the remainder of the scale was used, requiring assessment of at least two items. In the present sample the internal consistency of the scale was 0.70 at age two, 0.72 at age three, and 0.73 at age four.

Parental behavioural ratings from the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997) were obtained at the four-, seven-, and 12-year data collection points. The hyperactivity-inattention subscale of the SDQ is similar to the RRPSPC scale, in that it has a three-point scale and contains three overlapping (but slightly differently worded) items. In total there are five items (“restless, overactive, cannot stay still for long”; “constantly fidgeting or squirming”; “easily distracted, concentration wanders”; “thinks things out before acting” (reversed); “see tasks through to the end, good attention span” (reversed)). The total ADHD symptom score was calculated from the total sum of scores for each rated item, with a greater score indicative of higher ADHD symptoms. In a few cases missing responses to individual items were pro-rated: a summary score based on the mean of individual items of the remainder of the scale was used, requiring assessment of at least three items. The internal consistency of the scale was 0.76 at each of the three time-points.

When twins were aged, on average, eight and 12 years, parents were asked to rate the behaviour of each twin using the Revised Conners’ Parent Rating Scale (CPRS-R) (Conners et al., 1998a). The CPRS-R has two DSM-IV symptom sub-scales (inattentiveness and hyperactivity-impulsivity), each consisting of nine items that map onto DSM-IV criteria (see Table 1.1). The scale uses a four-point Likert scale: 0 indicates a response of ‘not true at all’, 1 indicates ‘just a little bit true’, 2 indicates ‘pretty much true’, and 3 indicates ‘very much true’. The sum of all 18 items calculates a total DSM-IV ADHD symptom score (values between 0 and 54), with a higher score indicating a greater endorsement of ADHD symptoms. In a few cases missing responses to individual items were pro-rated: a summary score based on the mean of individual items of the remainder of the scale was used, requiring assessment of at least nine items. The internal consistency of the CPRS-R was 0.93 at both ages.

2.3.2.2 Socio-demographic factors

Child's gender: Gender was re-coded so that females, which constituted the largest proportion of the total sample, were assigned as the reference group (0). (See Table 2.1 for breakdown of socio-demographic variables by sex-specific zygosity groups).

Family size: At the point of initial contact, when twins were aged 18 months and parents consented to participate in TEDS, parents were asked to provide information relating to the family. Parents were asked “how many other children live in the home with your twins?” Using this information a continuous variable relating to family size was created, to act as a proxy for exposure to children, based on the total number of *additional* children in the household.”

Child's ethnicity: Parents were asked to nominate twins' ethnicity based on a choice of five broad ethnic categories that were used in the 1991 UK Census: White, Black, Asian, Other and Mixed, which have been shown to map onto the more detailed 16 ethnic categories used in the UK 2001 Census (Kumarapeli, Stepaniuk, de Lusignan, Williams, & Rowlands, 2006). The vast majority of the sample had parent-nominated ethnicity: only 22 twin pairs have no recorded ethnicity. Out of 8748 twin pairs with ethnicity data, 93% had their ethnicity assigned as White (n=8135 twin pairs); 3% assigned Mixed (n=262); 1.95% assigned Asian (n=171); 1.36% assigned Black (n=119); and 0.70% assigned Other (n=61). Small samples across minority ethnic groups led to them being collapsed to produce one category (n=613). Ethnicity categories were re-coded as 1 or 0, such that 0 indicated the group with the larger sample size. Accordingly the white group was coded as 0, and the minority ethnic group coded as 1.

Socio-economic status: Socio-economic status (SES) was measured using demographic information collected at initial contact (when twins were 18 months old), and was missing for 8% of the entire sample (n=711 twin pairs). An index of SES was used based on a factor analysis of maternal and paternal occupational status and highest educational attainment (Pike, Iervolino, Eley, Price, & Plomin, 2006). Age of mother at the birth of her eldest child was also included as an indicator of low SES, as it loaded on the same factor. These five SES indicators were standardised, and then summed using unit weights in order to create a general single composite measure of SES, with a lower value representing a higher level of risk of low SES (Pike et al., 2006).

Table 2.1 Number of twin pairs by zygosity and gender, by socio-demographic variables

	Additional Children											Ethnicity		SES
	None	1	2	3	4	5	6	7	8	9	10	White	Ethnic Minority	SES recorded
MZM	656	527	188	64	9	1	1	1	1	0	0	1342	102	1327
MZF	730	592	217	54	18	0	3	1	1	0	0	1482	129	1467
DZSSM	689	490	187	42	17	5	1	0	0	0	0	1340	88	1334
DZSSF	696	466	193	73	15	7	0	0	0	0	0	1348	100	1341
DZOS	1396	917	354	122	29	4	2	0	0	0	1	2623	194	2590
TOTAL	4167	2992	1139	355	88	17	7	2	2	0	1	8135	613	8059

Note: Abbreviations- MZM: monozygotic males; MZF: monozygotic females; DZSSM: dizygotic same-sex males; DZSSF: dizygotic same-sex females; DZOS: dizygotic opposite-sex; SES: socio-economic status;

2.3.3 Analyses

In accordance with standard quantitative genetic procedures, transformations were applied to normalise the positively skewed distributions for ADHD symptom score ratings derived from the CPRS-R at ages 8 and 12, using the optimised minimal skew 'lnskew0' command in STATA (Stata, 2005). We then adopted a step-wise procedure to test for contrast effects.

2.3.3.1 Step 1: Testing variance differences according to zygosity to indicate possible contrast effects (twin correlation model without sibling interaction parameters)

In the first series of models, before modelling contrast effects, MZ and DZ twin correlations and variance estimates for non-adjusted parental ratings were obtained. Significance of variance differences between MZ and DZ (including DZ opposite-sex) pairs was evaluated by likelihood ratio testing, comparing a sub-model where variances were constrained to be equal across zygosity to one in which they were freely estimated across zygosity. Variance inequality tests were run separately for males and females to determine if gender moderated rater contrast effects. If findings were not consistent with the presence of contrast effects, these variables were dropped from subsequent analyses.

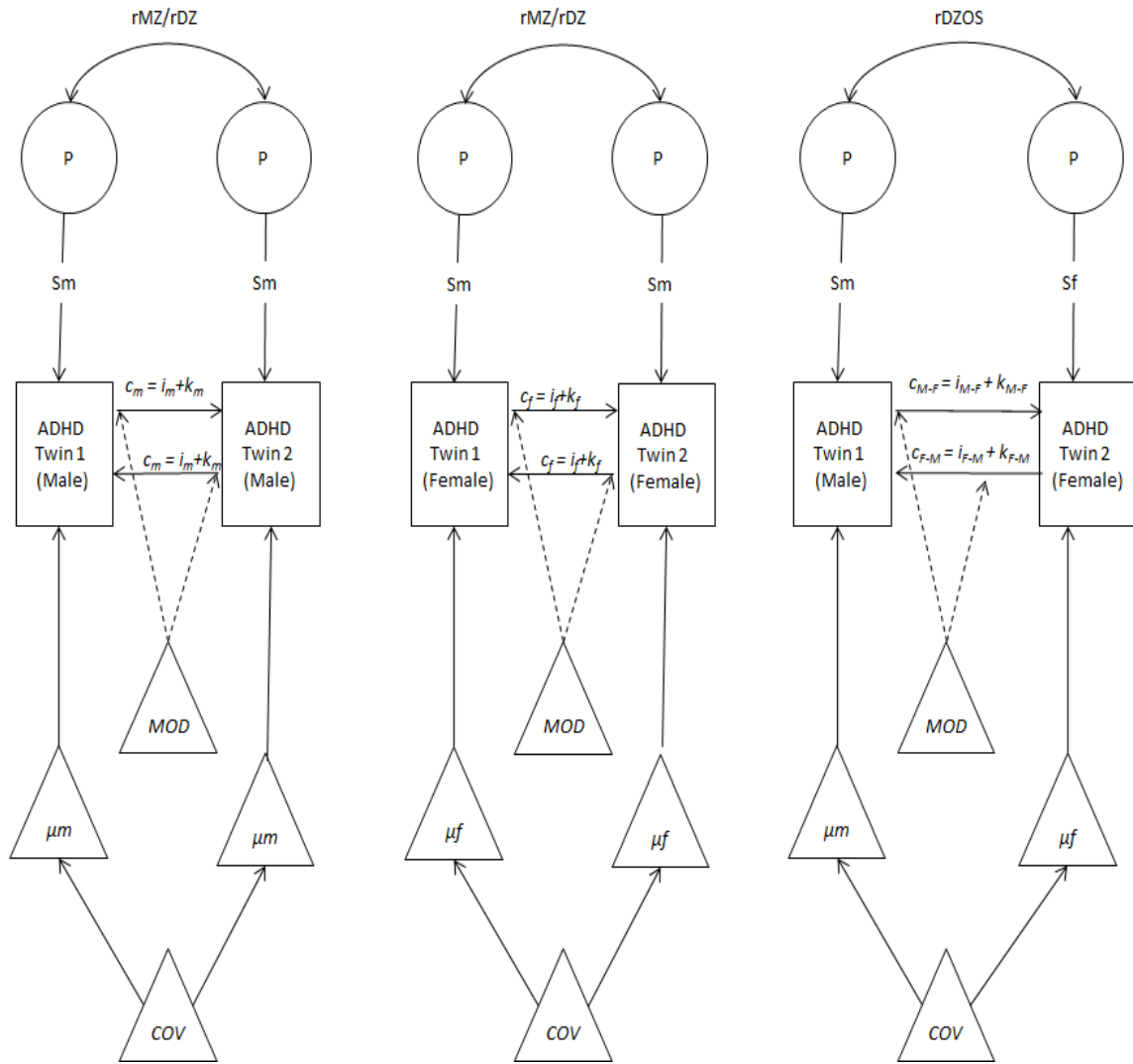
2.3.3.2. Step 2: Testing if contrast effects significantly differ between same-sex and opposite-sex pairs according to gender (twin correlation model with total sibling interaction parameter)

2.3.3.2.1 The rater contrast model

A non-genetic model was used to test rater contrast effects and the potential explanation of the socio-demographic factors on this effect. The model specified the variance-covariance structure of the MZ and DZ data as a $(I-B)^{inv} * (S*R*S') * ((I-B)^{inv})'$. The $S*R*S'$ part is a Gaussian decomposition of the variance-covariance structure of the data, where S is a 2 x 2 diagonal matrix with the standard deviations of the twin 1 and twin 2 scores of the phenotype under study, regardless of twin order and zygosity but sex specific (S_M and S_F), and R is a 2 x 2 correlation matrix between twin 1 and twin 2 score, estimating just one for MZ pairs (constrained across males and females) and one for DZ pairs (constrained across same-sex and opposite-sex pairs). The model further allowed for sex specific means (μ_M and μ_F). These

specifications are mainly based on previous findings of the same sample indicating that in parental ADHD ratings there are no quantitative or qualitative sex differences, but consistent evidence for sex differences in variance (Greven, Rijdsdijk et al., 2011; McLoughlin et al., 2007; Price et al., 2005; Price et al., 2001; Saudino et al., 2005). The rater contrast part is modelled in the (I-B) structure and is a standard way of specifying reciprocal causation pathways between two internal variables in structural equation models to overcome infinite feedback loops, where I is a 2 x 2 identity matrix and B is a 2 x 2 matrix, with zeros on the diagonals and symmetric off-diagonal parameters representing the reciprocal causal paths (the c paths in Figure 2.1).

In the second stage of analysis (disregarding the socio-demographic explanatory factors), four sex-by-zygosity rater contrast effect parameters were estimated to capture the possibility that different rater contrast effects may be present in pairs with varying composition of sex (see Figure 2.1): male-to-male (c_m), female-to-female (c_f), male-to-female (c_{M-F}) and female-to-male (c_{F-M}). The power to detect c_m and c_f is due to the fact that in this model the predicted variances and covariances will differ across MZ and DZ same-sex pairs. The power to detect c_{F-M} is based on observed differences in variance between same-sex males and DZ opposite-sex males. The power to detect c_{M-F} is based on observed differences in variance between same-sex females and DZ opposite-sex females. Age and all other moderators (family size, SES, and ethnicity) were incorporated as covariates in the model of the means (effectively regressing out any confounding effects). A series of sub-models were run to test whether c_m and c_{M-F} could be equated, and whether c_f and c_{F-M} could be equated, for the final stage of analysis.

Figure 2.1 The variance-covariance model of MZ/DZ twin pairs by varying gender composition

Note: The variance-covariance model of MZ, DZ same-sex pairs for males (model a), females (model b) and DZ opposite-sex pairs (model c); The correlation between scores are estimated separately for MZ and DZ pairs, but specified to be the same across genders in MZ pairs, and in DZ twins to be the same across opposite- and same-sex pairs; P is the phenotypic variation; S is the standard deviation of the ADHD symptom scores (S_M for males, S_F for females). The reciprocal causal paths between the phenotypes (c) are composed of a part independent of the specifically modelled moderators, indicated by i , and a moderator specific part (indicated by k). These effects differ according to gender composition of pairs, and are modelled separately for males ($_m$), females ($_f$), and for males-to-females ($_{M-F}$) and females-to-males ($_{F-M}$). MOD are definition variables modelling the moderator effect of ethnicity, SES and family size on the interaction terms (in step 3), and COV are definition variables modelling the effects of covariates in the model of the means (μ_M for males, and μ_F for females)

2.3.3.3 Step 3: Testing moderators of contrast effects (twin correlation model with independent and moderator-dependent sibling interaction effects)

Using the full form of the rater contrast model (see Figure 2.1), certain demographic variables of interest were incorporated to explore the extent to which they contributed to contrast effects. This involves splitting up the ‘total contrast effect’ (c_m , c_f , c_{M-F} , and c_{F-M}) into a moderator-independent part (i_m , i_f , i_{M-F} , and i_{F-M}) and a moderator-dependent part (k_m , k_f , k_{M-F} , and k_{F-M}), which is estimated by means of moderators on the interaction paths, incorporated as definition variables in Mx (Neale, Boker, Xie, & Maes, 2006). If findings from the second stage of analysis suggest that rater contrast parameters can be equated within gender groups (i.e. $c_m = c_{M-F}$; $c_f = c_{F-M}$), then only two sex-specific contrast effect parameters would be specified, and moderators included on these pathways. If not, four sex-by-zygosity contrast effect parameters would be specified (c_m , c_f , c_{M-F} , and c_{F-M}), and moderators modelled on each pathway. The power to detect c_m and c_f is due to the fact that in this model the predicted variances and covariances will differ across MZ and DZ same-sex pairs. The power to detect c_{F-M} is based on observed differences in variance between same-sex males and DZ opposite-sex males. The power to detect c_{M-F} is based on observed differences in variance between same-sex females and DZ opposite-sex females. Age was further incorporated as a covariate in the model of the means.

The structural equation-modelling program Mx (Neale, Boker et al., 2006) was used to conduct the analyses. Participants with missing data were included in the analyses, as Mx provides a method for handling incomplete data by using raw maximum likelihood estimation, in which a likelihood statistic (-2LL) for each observation is calculated. This implies that there is no overall measure of fit. Instead, with raw data, there are relative measures of fit: by comparing the -2LL of nested models a chi-square goodness-of-fit index (χ^2) is obtained, relative to a change in degrees of freedom (df). We adopted a p value of 0.01 to control for multiple testing.

2.4 Results

Means and variances by zygosity groups for all rating scales across all assessment points are presented in Table 2.2.

Table 2.2 Means (and variances) by sex-zygosity groups

ADHD scale (age)	SS				DZOS	
	MZM	DZM	MZF	DZF	Male	Female
RRPSPC (2)	3.00 (3.23)	3.01 (4.08)	2.71 (3.20)	2.78 (3.93)	3.10 (3.79)	2.53 (3.61)
RRPSPC (3)	2.92 (3.69)	2.86 (4.11)	2.55 (3.28)	2.67 (3.83)	3.05 (4.11)	2.30 (3.31)
RRPSPC (4)	2.84 (3.66)	2.80 (4.00)	2.32 (2.95)	2.43 (3.68)	2.92 (3.82)	2.04 (3.18)
SDQ (4)	4.34 (5.11)	4.17 (5.95)	3.71 (4.26)	3.76 (5.54)	4.36 (5.59)	3.30 (4.73)
SDQ (7)	3.99 (6.59)	3.90 (6.78)	3.18 (5.30)	3.34 (6.10)	4.13 (7.06)	2.89 (5.55)
SDQ (12)	3.36 (5.06)	3.23 (5.70)	2.29 (3.83)	2.50 (4.56)	3.48 (6.18)	2.14 (3.54)
CPRS-R (8)	12.68 (89.49)	12.72 (94.13)	9.49 (67.69)	9.47 (67.36)	12.94 (101.73)	8.65 (57.52)
CPRS-R (12)	11.46 (78.63)	11.31 (87.25)	8.11 (54.86)	8.55 (59.71)	11.92 (87.53)	7.59 (46.97)

Note: Abbreviations- SS: same-sex twin pairs; DZOS: dizygotic opposite-sex twin pairs; MZM: monozygotic male; DZM: dizygotic male; MZF: monozygotic female; DZF: dizygotic female; RRPSPC refers to Revised Parent Rutter Scale for Pre-School Children; SDQ refers to Strengths and Difficulties Questionnaire; CPRS-R refers to the Revised Conners' Parent Rating Scale;

2.4.1 Testing variance differences according to zygosity to indicate possible contrast effects

Twin correlations and tests for variance differences by zygosity were examined to determine whether there was evidence of contrast effects in parental ADHD ratings (see Table 2.3). Twin correlations for all RRPSPS and SDQ ratings were consistent with the presence of rater contrast or dominant genetic effects. Conversely, DZ correlations for CPRS-R ratings were greater than half of MZ correlations. Formal testing of zygosity differences in phenotypic variance by gender confirmed that contrast effects were not present for CPRS-R ratings. Consequently, CPRS-R ratings were not included in further analysis.

Evidence of contrast effects was found for all RRPSPS and SDQ ratings for female twins, but only for RRPSPS ratings at age two and SDQ ratings at age four in males. Although these findings suggest potential moderating effects of gender on rater contrast effects, the model did not take into account differences between DZ opposite-sex and DZ same-sex twin pairs. Therefore we did not exclude from further analysis male ratings which did not show evidence of contrast effects.

2.4.2 Testing if contrast effects significantly differ between same-sex and opposite-sex pairs according to gender

In this series of models the moderating effects of gender composition on total contrast effects was tested. There was evidence to suggest that contrast effect parameters could be equated between same-sex males and opposite-sex males, and between same-sex females and opposite-sex female twins, for RRPSPS ratings at age two and SDQ ratings at age seven (see Table 2.4). However, overall the evidence suggested that the interaction pathways between same-sex and opposite-sex pairs were significantly different across both males and females, and that equating these parameters would result in a significant deterioration of fit.

Table 2.3 Twin correlations and variance estimates by zygosity: testing zygosity differences in variances

	Correlations		Variances				Test if vMZ = vDZ (χ^2 (1 df)) ^d	
ADHD scale (age)	rMZ	rDZ ^a	vMZM	vDZM ^b	vMZF	vDZF ^c	Males	Females
RRPSPC (2)	0.65	0.18	3.21	3.85	3.22	3.93	14.00 (p < 0.001)	18.53 (p < 0.001)
RRPSPC (3)	0.64	0.03	3.64	3.91	3.30	3.91	2.22 (p = 0.14)	13.78 (p < 0.001)
RRPSPC (4)	0.58	-0.02	3.55	3.84	3.03	3.69	3.93 (p = 0.05)	25.89 (p < 0.001)
SDQ (4)	0.50	-0.07	5.04	5.67	4.32	5.51	8.99 (p = 0.003)	41.09 (p < 0.001)
SDQ (7)	0.57	-0.03	6.49	6.69	5.36	6.42	0.63 (p = 0.43)	24.64 (p < 0.001)
SDQ (12)	0.74	0.18	5.10	5.48	3.80	4.92	2.63 (p = 0.11)	37.94 (p < 0.001)
CPRS-R (8)	0.86	0.47	0.34	0.36	0.32	0.35	1.70 (p = 0.19)	3.98 (p = 0.05)
CPRS-R (12)	0.86	0.45	0.62	0.41	0.38	0.40	1.94 (p = 0.16)	1.69 (p = 0.16)

Note: RRPSPC refers to Revised Parent Rutter Scale for Pre-School Children; SDQ refers to Strengths and Difficulties Questionnaire; CPRS-R refers to the Revised Conners' Parent Rating Scale; CPRS-R ratings were positively skewed and normalised prior to analysis; MZ refers to monozygotic; DZ refers to Dizygotic; rMZ and rDZ refer to twin correlations; vMZM and vMZF refer to MZ variance estimates for males and females, respectively; vDZM and vDZF refer to DZ variance estimates for males and females; ^a includes DZ opposite-sex (OS) pairs; ^b includes DZOS males; ^c includes DZOS females; ^d Compared a model where variances constrained across zygosity (1 df); Significant variance differences indicated by a p value in bold typeface; a p value of 0.01 was adopted to control for multiple testing.

Table 2.4 Contrast effect parameters between same-sex and opposite-sex pairs, by gender

	Males			Females		
ADHD scale (age)	c_m	c_{M-F}	Test if $c_m = c_{M-F}$ (χ^2 (1 df)) ^a	c_f	c_{F-M}	Test if $c_f = c_{F-M}$ (χ^2 (1 df)) ^a
RRPSPC (2)	-0.19	-0.10	2.31 (p = 0.13)	-0.20	-0.23	0.38 (p = 0.54)
RRPSPC (3)	-0.14	0.08	7.17 (p = 0.007)	-0.16	-0.47	9.74 (p = 0.002)
RRPSPC (4)	-0.18	0.008	6.02 (p = 0.01)	-0.19	-0.41	6.88 (p = 0.009)
SDQ (4)	-0.22	0.02	7.31 (p = 0.007)	-0.22	-0.48	7.06 (p = 0.008)
SDQ (7)	-0.11	0.03	1.60 (p = 0.21)	-0.13	-0.32	1.75 (p = 0.19)
SDQ (12)	-0.27	-0.15	12.66 (p < 0.000)	-0.25	-0.52	37.58 (p < 0.001)

Note: Abbreviations- RRPSPC: Revised Parent Rutter Scale for Pre-School Children; SDQ: Strengths and Difficulties Questionnaire; CPRS-R ratings were not included in analysis as they showed no significant variance differences by zygosity in initial stage of analysis, suggesting the absence of contrast effects; c_m : contrast effect parameter between males; c_f : contrast effect parameter between females; c_{M-F} : contrast effect parameter from males-to-females; c_{F-M} : contrast effect parameter from females-to-males; ^a Compared a model where contrast effect parameters constrained across same-sex and opposite-sex pairs, for males and females separately (1 df); Contrast effect parameters that significantly differed between gender-composition of twin pairs (i.e. same-sex versus opposite-sex) indicated by a p value in bold typeface (a p value of 0.01 was adopted to control for multiple testing);

2.4.3 Testing moderators of contrast effects

Due to the lack of consistent evidence for equating same-sex and opposite-sex contrast effects parameters by gender (section 2.4.2), subsequent models testing for the contributory role of demographic factors on contrast effects specified four separate parameters (c_m , c_f , c_{M-F} , and c_{F-M}). Accordingly, these models tested the moderating effects of gender on rater contrast effects in general (independent component), and the interactive effects between gender and other moderators (family size, SES, and ethnicity) (moderator-dependent component).

2.4.3.1 Moderating effects of gender

The independent components of contrast effect parameters were significant for same-sex pairs, across both genders (see Table 2.5). Although the effect was always larger for female same-sex pairs, they did not significantly differ from estimates for male same-sex pairs (overlapping confidence intervals (CIs)). From age three a gender effect was observed within DZ opposite-sex pairs: the independent component of the interaction parameter was not significant from males-to-females, but significant from females-to-males. The independent component of the contrast effect from females-to-males was larger than observed for same-sex pairs, but as CIs overlapped the magnitude of the difference was not statistically significant.

2.4.3.2 Interactive effect of gender and other socio-demographic moderators on contrast effects

When partitioning the contrast effect components moderated by SES or ethnicity, there was no evidence to suggest that these demographic variables contributed significantly to contrast effects, or that their effect was moderated by gender (see Table 2.5). Family size did not contribute to significant contrast effects in male same-sex pairs or DZ opposite-sex pairs. However, the family size dependent interaction parameter was small, but significant, in female same-sex pairs from age four. Family size was a continuous variable, and the effect suggests that as family size increases the contrast effect parameter increases alongside. The positive value for these significant contrast effect parameters suggest that when combined with the negative contrast effect parameters found for independent contrast effect component, total contrast effects are reduced. Therefore parental ratings of twins from larger families are associated with smaller (overall) rater contrast effects.

Table 2.5 Contrast effect parameters (and 99% confidence intervals) by gender composition of twin pairs, decomposed into independent and moderator-dependent components

	ADHD scale (age)					
	RRSPC (2)	RRSPC (3)	RRSPC (4)	SDQ (4)	SDQ (7)	SDQ (12)
Independent						
C_m	-0.19 (-0.42/-0.05)	-0.14 (-0.25/-0.04)	-0.19 (-0.32/-0.10)	-0.25 (-0.36/-0.15)	-0.17 (-0.26/-0.18)	-0.27 (-0.44/-0.15)
C_f	-0.19 (-0.42/-0.06)	-0.18 (-0.29/-0.08)	-0.24 (-0.37/-0.15)	-0.31 (-0.42/-0.21)	-0.18 (-0.27/-0.09)	-0.29 (-0.46/-0.16)
C_{M-F}	-0.16 (-0.43/0.13)	0.04 (-0.24/0.23)	-0.12 (-0.32/0.07)	-0.12 (-0.35/0.06)	0.02 (-0.19/0.17)	-0.16 (-0.36/0.02)
C_{F-M}	-0.23 (-0.46/0.04)	-0.48 (-0.68/-0.11)	-0.36 (-0.57/-0.09)	-0.42 (-0.63/-0.12)	-0.37 (-0.59/-0.08)	-0.50 (-0.68/-0.32)
Dependent						
Family size						
C_m	0.005 (-0.04/0.05)	-0.0008 (-0.04/0.04)	-0.0001 (-0.04/0.04)	0.01 (-0.02/0.05)	0.04 (-0.01/0.07)	0.03 (-0.01/0.06)
C_f	0.002 (-0.03/0.04)	0.01 (-0.02/0.05)	0.06 (0.03/0.09)	0.08 (0.05/0.11)	0.03 (0.01/0.06)	0.04 (0.01/0.07)

c_{M-F}	0.07 (-0.06/0.19)	0.02 (-0.18/0.18)	0.13 (-0.002/0.25)	0.11 (-0.02/0.24)	0.02 (-0.13/0.15)	0.02 (-0.06/0.09)
c_{F-M}	0.001 (-0.11/0.12)	0.02 (-0.14/0.21)	-0.05 (-0.17/0.08)	-0.04 (-0.17/0.10)	0.02 (-0.02/0.18)	-0.03 (-0.11/0.06)
SES						
c_m	-0.02 (-0.07/0.02)	-0.02 (-0.07/0.02)	-0.02 (-0.06/0.03)	-0.01 (-0.06/0.03)	0.02 (-0.02/0.06)	-0.001 (-0.04/0.04)
c_f	0.004 (-0.04/0.05)	0.03 (-0.01/0.08)	0.006 (-0.03/0.05)	0.02 (-0.02/0.06)	0.02 (-0.01/0.06)	0.002 (-0.04/0.04)
c_{M-F}	-0.03 (-0.19/0.15)	0.007 (-0.16/0.18)	-0.07 (-0.25/0.11)	0.05 (-0.11/0.19)	0.15 (-0.01/0.28)	-0.04 (-0.13/0.06)
c_{F-M}	-0.004 (-0.17/0.16)	-0.01 (-0.19/0.17)	0.06 (-0.15/0.26)	-0.07 (-0.24/0.11)	-0.06 (-0.21/0.12)	0.02 (-0.11/0.14)
Ethnicity						
c_m	-0.04 (-0.20/0.12)	-0.06 (-0.21/0.10)	0.05 (-0.10/0.19)	0.05 (-0.08/0.19)	0.08 (-0.05/0.21)	0.07 (-0.07/0.21)
c_f	-0.07 (-0.21/0.07)	0.09 (-0.06/0.24)	-0.02 (-0.14/0.11)	-0.006 (-0.13/0.12)	0.08 (-0.19/0.03)	-0.03 (-0.15/0.09)
c_{M-F}	0.04 (-0.41/0.46)	-0.09 (-0.74/0.47)	-0.39 (-0.82/0.44)	0.22 (-0.57/0.57)	-0.01 (-0.71/0.34)	-0.08 (-0.36/0.16)

c_{F-M}	0.10	0.32	0.50	-0.13	-0.05	0.08
	(-0.31/0.55)	(-0.28/0.99)	(-0.47/0.95)	(-0.54/0.69)	(-0.43/0.63)	(-0.24/0.40)

Note: RRPSPC refers to Revised Parent Rutter Scale for Pre-School Children; SDQ refers to Strengths and Difficulties Questionnaire; CPRS-R ratings were not included in analysis as they showed no significant variance differences by zygosity in initial stage of analysis, suggesting the absence of contrast effects; c_m refer to contrast effect parameter between males; c_f refer to contrast effect parameter between females; c_{M-F} refer to contrast effect parameter from male-to-female; c_{F-M} refer to contrast effect parameter from female-to-male; significant contrast effects (i.e. 99% CI not overlapping with the value zero) are indicated by bold typeface;

2.5 Discussion

This study employed a novel twin model to explicitly test for factors moderating contrast effects in parental ADHD ratings. This was achieved using a detailed examination of several socio-demographic variables in a large population-based twin sample, and partitioning the total contrast effects observed into independent and moderator-dependent components. Moreover, the inclusion of opposite-sex twins, allowed us to not only test the moderating and interactive role of gender across same-sex pairs, but also to test whether gender plays a differing role within opposite-sex twins.

The first main finding was that within same-sex twin pairs parents contrast twins to a similar extent, regardless of whether they are female or male (independent component). Furthermore, there was a gender effect within opposite-sex pairs: rater contrast parameters were significant only when the pathway was specified from females-to-males (from age three), suggesting that parents evaluate males in relation to their female co-twin, who is considered the standard. The non-significant contrast effect parameters from males-to-females suggests that when female members of an opposite-sex pair are being rated, their evaluation is being made independent of their male co-twin's behaviour. This was an unexpected finding, as the only previous study to distinguish contrast effects within DZ opposite-sex pairs observed a larger effect from males-to-females (Rietveld, Hudziak et al., 2003). The original direction of the effect was hypothesised to stem from the assumption that these behaviours are more commonly associated with males, and so males are considered the comparative benchmark, and females rated according to their male co-twin. However, if there is a greater awareness of normative standards for ADHD-related behaviours in males, one may also expect contrast effects to feature less in parental ratings of male versus female same-sex pairs. Yet findings from this study suggest that there are no differences in the magnitude of contrast effects across genders within same-sex pairs, in line with previous research (Rietveld, Hudziak et al., 2003; Simonoff et al., 1998). On the contrary, we speculate that our findings could be interpreted as suggesting that parents of opposite-sex pairs are more acutely aware of behavioural differences (actual and/or stereotypical). Accordingly, when female members of opposite-sex pairs are being rated by parents, their evaluation is being made independent of their male co-twins behaviour (as suggested by our non-significant contrast effect parameter from males-to-females). This finding

was consistent across ages and rating scales (with the exception of the first assessment point). However, it is important to note that this is the first attempt at a replication of the original finding and so further studies are needed to clarify the direction of the effect, and it may be that this observed discrepancy is based on sample differences and/or the use of different assessment instruments.

Our second key finding was that family size did moderate contrast effects, in line with previous predictions (Carey, 1986), and in the expected direction: parental ratings where family size was small were more likely to result in overall larger contrast effects. However, this effect was only observed for female same-sex pairs. The finding that contrast effects featured less in parental ratings of larger sized families might be explained by increased awareness of a broader range of child behaviours in these families, and a larger baseline for comparing behaviour. Parents of smaller sized families are more likely to have a smaller benchmark to make comparisons with, and are therefore more likely to directly compare twins. This finding is consistent with the hypothesis that contrast effects do not feature in teacher ratings of ADHD-related behaviours as they have greater exposure to children and more objective standards of appropriate behavioural norms (Hartman et al., 2007; Saudino et al., 2005). The fact that the moderating effect of family size on contrast effects was only evident in females, could relate to ADHD-related behaviours being less commonly associated with females and consequently there being less clearly defined norms for these behaviours in girls; such that parents draw on a broader comparative group where possible (i.e. where they have a large family). However, the magnitude for the majority of the family-size dependent contrast effect parameters is similar across male and female same-sex pairs, and likely reaches significance in females due to a greater number of female same-sex twin pairs in our sample. If we eliminate such cases the most robust gender difference for the effect of family-size on the interaction parameter is found at age four, independent of rating scale.

We replicated previous findings that not all parent rating scales are equally susceptible to rater bias, and that the Conners' Parent Rating Scale is particularly robust against contrast effects, confirming previous observations that particularly implicate short general rating scales (Price et al., 2005; Price et al., 2001), rather than longer more detailed scales such as DSM-IV checklists (Kuntsi, Rijdsdijk et al., 2005). This may be attributable to the broader scoring range and/or more

detailed and precise nature of the items being rated, compared to the other scales examined. This is consistent with the observation that the Strengths and Weaknesses of ADHD Symptoms and Normal Behaviors (SWAN) scale (Swanson et al., 2006), which uses similar detailed questions and a seven-point scoring range to measure both positive and negative behaviours, seems immune to contrast effects (Hay et al., 2007; Polderman et al., 2007). Another possibility is that the Conners' Parent Rating Scale with 18 items that parallel current DSM-IV criteria may be measuring a partially different behavioural trait compared to the other shorter rating scales used in this study. However, it may also be the case that the lack of contrast effects in ratings from the Conners' Parent Rating Scale obtained at ages eight and 12, reflect an a developmental decline in contrast effects. We were unable to test this directly, as we did not have ratings from this scale at younger ages. However, comparison of cross-sectional analysis of SDQ ratings in TEDS suggest a developmental decline in contrast effects (Merwood et al., Submitted), which was also reported in an independent twin sample with maternal CBCL ratings (Rietveld, Hudziak et al., 2003).

The other socio-demographic factors investigated (ethnicity and SES) did not contribute to parental rater contrast effects. Despite methodological and sample differences, our finding that SES did not contribute to contrast effects is in line with previous findings in a non-twin sample (Saudino et al., 2004). In order to potentially control for rater contrast effects and improve rating scales, further research is needed to clarify the origins of contrast effects. For example, societal factors may be important and variations in contrast effects in parental ratings from collectivist versus individualistic societies could be examined (Saudino et al., 2004).

A limitation of this study is that we did not have information on parental ethnicity. Consequently we used childrens' ethnicity as a marker, albeit less accurate, of parental ethnicity, to test for cultural differences in parents' tendency to contrast twins. A further limitation is that our family size variable was collated from information when twins were aged 18 months, and could not have taken into account additional children previously or subsequently residing in the household. Taking these factors into account, replication is needed before firm conclusions can be made.

There is only one other study that we are aware of to test whether parental demographics contributed to contrast effects in ADHD-related ratings, and this was carried out using correlational analysis between difference scores on parental ratings and parental demographics in a small non-twin sibling sample (Saudino et al., 2004). Ideally contrast effects can be formally tested using structural equation modelling, and our study employed a particularly novel methodology to disentangle contrast effects to determine underlying sources. An important consideration for future research is sample size, as there is limited power to detect contrast effects in small twin samples, especially when genetic dominance effects may be present (Rietveld, Posthuma et al., 2003), as has been reported in a meta-analysis of ADHD behaviours (Burt, 2009). Consequently, our large sample size was a major strength in this study.

Contrast effects in parental ratings of ADHD symptoms clearly need to be acknowledged as a potential bias in quantitative genetic research, which may have contributed to over-estimated heritability estimates, which is an important consideration for phenotype selection in molecular genetic investigations. In addition, it may be the case that inflated heritability estimates have contributed to less attention being directed at environmental factors underlying ADHD presentation. Our study identified family size as a significant contributor to contrast effects, from the socio-demographic variables investigated. This finding is consistent with the view that contrast effects reflect parental rating biases, rather than actual behavioural differences, as phenotypic differences in the presentation of ADHD-related behaviours are unlikely to vary according to family size. Overall, this and previous studies (Sherman, McGue et al., 1997; Thapar et al., 2000) support the use of multiple informants in studies on ADHD, and further indicate that selection of rating scale does matter and that studies should therefore also endeavour to use measurement scales that are less susceptible to contrast effects. In addition, research directed at obtaining more objective cognitive, metabolic or neurological ADHD markers will help overcome the reliance on ADHD symptom scales and the biases they may be associated with.

CHAPTER 3 THE SEPARATION OF ADHD INATTENTION AND HYPERACTIVITY-IMPULSIVITY SYMPTOMS: PATHWAYS FROM GENETIC EFFECTS TO COGNITIVE IMPAIRMENTS AND ADHD SYMPTOMS

3.1 Abstract

Both shared and unique genetic risk factors underlie the two symptom domains of attention-deficit/hyperactivity disorder (ADHD): inattention and hyperactivity-impulsivity. The developmental course and relationship to co-occurring traits differs across the two symptom domains, highlighting the importance of their partially distinct aetiologies. Two familial cognitive impairment factors have been identified in ADHD, but whether they show specificity in relation to the two ADHD symptom domains remains poorly understood. A better understanding of the underlying risk pathways is required for the development of targeted interventions. This study aimed to determine whether there is evidence of separate aetiological pathways, by investigating if distinct ADHD-related familial cognitive impairments are differentially genetically linked to the ADHD symptom domains of inattention versus hyperactivity-impulsivity. Multivariate structural equation modelling was conducted on cognitive-experimental measures derived from a four-choice reaction time (RT) task and a go/no-go (GNG) inhibition task and ADHD behavioural ratings obtained on a population-based twin sample of 1312 children aged seven to 10. Reaction time variability (RTV) showed substantial genetic overlap with inattention, as observed by a genetic correlation of 0.64, compared to a genetic correlation of 0.31 with hyperactivity-impulsivity. Commission errors (CE) showed low genetic correlations with both hyperactivity-impulsivity (0.17) and inattention (0.11). The genetic correlation between RTV and CE was also low and non-significant at -0.10, consistent with the familial separation of these two indices of cognitive impairments. In conclusion, two key cognitive impairments phenotypically associated with ADHD, captured by RTV and CE, showed different genetic relationships to the two ADHD symptom domains. Overall, the findings extend a previous model of two familial cognitive impairment factors in combined ADHD (ADHD-C) subtype, by separating pathways underlying inattention and hyperactivity-impulsivity symptoms.

3.2 Introduction

Two behavioural symptom domains underlie the current conceptualisation of attention-deficit/hyperactivity disorder (ADHD): inattention and hyperactivity-impulsivity (American Psychiatric Organisation, 2000). Previous twin analyses on ADHD symptom scores indicate that 55-83% of the genetic influences on inattention also influence hyperactive-impulsivity, with the remaining genetic influences reflecting those that are unique to each symptom domain (Greven, Asherson et al., 2011; McLoughlin et al., 2011; McLoughlin et al., 2007; Schultz et al., 2006; Wood et al., 2009). Despite the substantial shared genetic component, converging evidence highlights the importance of the partially distinct aetiologies. For example, co-occurring neurodevelopmental and behavioural problems differ, with reading difficulties linked predominantly to inattention (Paloyelis et al., 2010; Willcutt et al., 2007), and oppositional behaviours to hyperactivity-impulsivity (Newcorn et al., 2001; Wood et al., 2009). Furthermore, hyperactivity-impulsivity decreases relative to inattention throughout development in both clinical (Biederman, Mick, & Faraone, 2000; Todd et al., 2008) and population (Larsson, Lichtenstein, & Larsson, 2006) samples.

The emerging knowledge of the shared and unique aetiological influences on the two ADHD symptom domains raises questions about how this maps onto cognitive impairments, particularly those that index the familial risk for ADHD. In a recent large-scale investigation of ADHD and control sibling pairs, we obtained evidence for two familial cognitive impairment factors in ADHD (Kuntsi et al., 2010). The larger familial factor, accounting for 85% of the familial variance of ADHD, captured 98-100% of the familial influences on mean reaction time (MRT) and reaction time variability (RTV) (Kuntsi et al., 2010). This factor separated from a second smaller familial factor that captured 62-82% of the familial influences on omission (OE) and commission errors (CE) on a go/no-go (GNG) task, and accounted for 13% of the familial variance of ADHD. Drawing on the arousal-attention (Johnson, Kelly et al., 2007; O'Connell et al., 2008) and developmental (Halperin & Schulz, 2006; Halperin et al., 2008) models of ADHD (see sections 1.5.2.2 and 1.5.3), we proposed that the first reaction time (RT) factor may represent bottom-up arousal dysregulation and the second factor (errors) top-down control of sustained attention and inhibition (Kuntsi et al., 2010). However, this study based on a clinical sample of probands with combined ADHD (ADHD-C) subtype, was unable to examine the

specificity that the cognitive impairment factors may have in relation to inattention and hyperactivity-impulsivity symptoms considered separately.

Previous comparisons of cognitive performance between predominantly inattention (ADHD-PI) and ADHD-C subtypes have failed to identify clearly distinguishable cognitive profiles (Carr, Henderson, & Nigg, 2010). Empirical approaches to ADHD subtypes indicate that many cases with ADHD-I reflect sub-threshold ADHD-C subtype, and should not be treated as a separate category (Todd et al., 2001). Furthermore, ADHD subtypes are unstable, with many individuals with ADHD-C subtype re-classified as ADHD-PI subtype as they grow older (Biederman et al., 2000). A more strictly defined pure inattentive subtype was, however, linked to early attentional problems and inconsistent performance, whereas inhibition deficits were observed across ADHD subgroups (Adams, Derefinko, Milich, & Fillmore, 2008; Carr et al., 2010).

The present study applies a multivariate twin model fitting approach on a population twin sample to investigate inattentive and hyperactive-impulsive symptoms separately. Using the twin sample, we previously found that associations between ADHD symptoms and the cognitive impairments of slow and variable RT and CE (Kuntsi et al., 2009; Wood, Asherson et al., 2010) were similar to those observed in a large clinical sample of ADHD-C subtype cases (Andreou et al., 2007; Kuntsi et al., 2010; Uebel et al., 2010; Wood, Rijdsdijk et al., 2011). In both samples we have recently also shown that RTV difference scores, which capture the ADHD-sensitive improvement in RTV (for example under rewarded conditions (Andreou et al., 2007; Kuntsi et al., 2009; Uebel et al., 2010)), measure largely the same aetiological process as RTV under baseline condition (Kuntsi et al., *in press*), supporting theories emphasising the malleability of the observed high RTV (see section 1.5.2).

We now address two new questions. First, using multivariate twin model fitting, we investigate the aetiological associations between the previously identified cognitive impairments and the two symptom domains of ADHD considered separately. Secondly, we consider whether the aetiological separation between impaired RT performance and CE in a clinical sample (Kuntsi et al., 2010), is confirmed in a population twin sample.

3.3 Methodology

3.3.1 Sample and procedure

Participants are members of the Study of Activity and Impulsivity Levels in children (SAIL), a general population sample of twins aged seven to 10 years. They were recruited from the Twins' Early Development Study (TEDS (Trouton et al., 2002)), a birth cohort study which invited parents of all twins born in England and Wales during 1994-1996 to enrol. The TEDS families are representative of the UK population with respect to parental occupation, education and ethnicity (Oliver & Plomin, 2007). Zygosity was determined using a parental questionnaire on physical similarity, which has demonstrated over 95% accuracy when compared with DNA testing (Price et al., 2000). For cases where zygosity was unclear from this standard zygosity questionnaire, DNA testing was conducted.

TEDS families were invited to take part if they fulfilled the following SAIL project inclusion criteria: twins' birthdates between September 1, 1995, and December 31, 1996; lived within a feasible travelling distance from the research centre; White European ethnic origin (to reduce population heterogeneity for molecular genetic studies); recent participation in TEDS, as indicated by return of questionnaires at either four- or seven-year data collection point; no extreme pregnancy, perinatal difficulties, specific medical syndromes, chromosomal anomalies or epilepsy; not participating in other current TEDS sub-studies; and not on stimulant or other neuropsychiatric medications.

Of the 1,230 suitable families contacted, 672 families (55%) agreed to participate. Overall, the sample is as representative of the general population as is feasible for a study of this kind. Moreover, previous analyses on TEDS indicated that attrition was unrelated to ADHD symptoms: twins who participated at age seven assessments did not significantly differ from non-participating twins, in hyperactivity parental ratings at age two ($t = 1.77$, $p = 0.08$) (Saudino et al., 2005). However a slight bias towards higher parental occupational classification, compared to the original TEDS sample was observed (39% of mothers and 52% of fathers in managerial or professional jobs, compared to 28% and 40%, respectively) (Saudino et al., 2005). 32 individual children were subsequently excluded due to: IQ < 70, epilepsy, obsessive-compulsive disorder, autism or other neurodevelopmental disorder, illness during testing or placement on stimulant medication for ADHD. The final sample consisted of 1312 individuals:

257 monozygotic (MZ), 181 full dizygotic (DZ) same-sex (SS) and 206 DZ opposite-sex (OS) complete twin pairs, as well as 24 singletons from three MZ, seven DZSS, and 14 DZOS pairs with one excluded twin member. Data for the singleton twins were also used in structural equation modelling (Neale, Boker et al., 2006). An additional three children had testing caution notes (very distressed on day of testing; did not appear to understand instructions; had hearing aid and had trouble hearing instructions), and also had outlier cognitive data (presumed to be as a result of testing issues outlined above (testing caution notes)), and so had all their cognitive data subsequently coded as missing). The number of pairs for each measure split by zygosity can be found in Table 3.1. The mean age of the sample was 8.83 years ($sd = 0.67$), and half of the sample were girls (51%).

Participants were invited to our research centre for cognitive assessment. Two testers assessed the twins simultaneously in separate testing rooms. The tasks were administered in a fixed order as part of a more extensive test session, which in total (including breaks) lasted approximately 2.5 hours. In addition while the families visited the research centre for assessments, behavioural ratings on the Conners' scale were collected from parents. Teachers' ratings on the Conners' scale were obtained through the post. Parents of all participants gave informed consent following procedures approved by the Institute of Psychiatry Ethical Committee.

3.3.2 Measures

3.3.2.1 Behavioural rating scales

Parents and teachers were asked to complete the Long Versions of Conners' Parent and Teacher Rating Scales (Conners et al., 1998a; Conners, Sitarenios, Parker, & Epstein, 1998b), rating each child's behaviour on a four-point Likert scale from (0) 'not true at all' to (3) 'very much true'. Ratings were completed by the primary caregiver, which for the majority was the mother. Teacher data were completed by the main class teacher for each child; previous analyses on the TEDS sample, from which the current subsample is drawn, indicates that the majority of twins are rated by the same teacher (Saudino et al., 2005). Teacher ratings were missing for 151 individuals and an additional two individuals did not have parent ratings. In a few cases, missing responses to individual items in the Conners' subscales were pro-rated: a

summary score based on the mean of individual questions on the remainder of the subscale was used if there was more than 75% completion for each subscale. From both scales, we used the nine-item inattention and nine-item hyperactivity-impulsivity DSM-IV ADHD symptom subscales, obtaining summed parent and teacher ratings on the corresponding subscales. Inter-rater agreement for parent and teacher ratings was moderate for both inattention ($r = 0.45$, $p < 0.001$) and hyperactivity-impulsivity ($r = 0.40$, $p < 0.001$), which is comparable to those obtained in previous studies (Saudino et al., 2005).

3.3.2.2 Wechsler Intelligence Scales for Children

The vocabulary, similarities, picture completion and block design subtests from the third edition of the Wechsler Intelligence Scales for Children (WISC-III) (Wechsler, 1991) were used to obtain an estimate of child's IQ (prorated following procedures described by (Sattler, 1992)).

3.3.2.3 The go-no/go task

On each trial of the GNG task (Borger & van der Meere, 2000; Kuntsi, Andreou et al., 2005; van der Meere, Stemerding, & Gunning, 1995) one of two possible stimuli appeared for 300 milliseconds in the middle of the computer screen. The child was instructed to respond only to the 'go' stimuli and to react as quickly as possible, but to maintain a high level of accuracy. The proportion of 'go' stimuli to 'no-go' stimuli was 4:1. The participants performed the task under three conditions (slow, fast and incentive), matched for length of time on task. Herein we present data from the slow condition, which had an inter-stimulus interval of eight seconds and consisting of 72 trials, and the fast condition, with an inter-stimulus interval of one second and consisting of 462 trials. The order of presentation of the slow and fast conditions varied randomly across participants. The variables obtained from the task are MRT, standard deviation of RTs (RTV), CE and OE.

3.3.2.4 The fast task

The baseline condition of the fast task (Andreou et al., 2007; Kuntsi, Andreou et al., 2005; Kuntsi, Rogers et al., 2006) with a fore period of eight seconds and consisting of 72 trials, followed a standard warned four-choice RT task (Leth-Steensen, Elbaz, & Douglas, 2000). A

warning signal (four empty circles, arranged side by side) first appeared on the screen. At the end of the fore period of eight seconds (presentation interval for the warning signal), the circle designated as the target signal for that trial was filled (coloured) in. The child was asked to make a compatible choice by pressing the response key that directly corresponded in position to the location of the target stimulus. After a response, the stimuli disappeared from the screen and a fixed inter-trial interval of 2.5 seconds followed. Speed and accuracy were emphasized equally. If the child did not respond within 10 seconds, the trial was terminated. A comparison condition with a fast event rate (one second) and incentives followed the baseline condition (Andreou et al., 2007). The variables obtained from the task are MRT and RTV, herein reported for the baseline condition.

3.3.2.5 Selection of cognitive variables for analyses

To limit the total number of variables, to create psychometrically robust variables (Kuntsi, Rogers et al., 2006) and to enable a comparison to our previous findings using the same tasks in a clinically diagnosed sample (Kuntsi et al., 2010), summed scores were obtained across two tasks or conditions as follows: unstandardised MRT and RTV across the slow condition of the GNG task and baseline condition of the fast task (MRT: $r = 0.41$, $p < 0.001$; RTV: $r = 0.31$, $p < 0.001$); and percentage of CE across slow and fast conditions of the GNG task ($r = 0.52$, $p < 0.001$). (As conditions differed according to number of trials, unstandardised scores across conditions were used). OE on the GNG task were rare in this population sample and therefore were not included, in line with previous analyses on this sample (Kuntsi, Rogers et al., 2006; Kuntsi et al., 2009).

Each summed composite cognitive variable was regressed for age, sex, and IQ. Although our previous analyses indicated that the majority of genetic influences shared between ADHD and cognitive variables were independent of those shared with IQ (Kuntsi et al., 2010; Wood, Rijdsdijk et al., 2011), regressing for IQ ensured we controlled for any small mediating effects of IQ that were not the focus of present analyses.

3.3.3 Analyses

3.3.3.1 Structural equation modelling

Structural equation modelling was performed using Mx (Neale, Boker et al., 2006). Models were fitted to age- and sex-regressed (cognitive variables had IQ additionally regressed) unstandardised residuals. All variables were positively skewed (1.06 to 1.92; except CE (-0.12)), and were transformed using the optimised minimal skew command 'lnskew0' in STATA, which reduces the skew statistic to 0 by using a log transformation together with an optimised constant (Stata, 2005).

Models were fitted using raw data analysis, rather than covariance matrices. The advantage of this approach is that participants with incomplete data and data from singletons (incomplete twin pairs) can be included in the analyses, as Mx provides a method for handling such missing data by using raw maximum likelihood estimation, in which a likelihood statistic (-2LL) of the data for each observation is calculated. This implies that there is no overall measure of fit (such as a χ^2 value with corresponding p value for the number of degrees-of-freedom (df), as obtained by fitting directly on observed variance-covariance matrices). Instead, with raw data, there are relative measures of fit: by comparing the -2LL (and df) of the saturated model (where the maximum number of parameters is estimated to describe the correlational structure between variables), with the -2LL (and df) of genetic models. This provides a likelihood ratio chi-square test of goodness of fit. The difference between the measure of fit of the saturated model and the genetic model is distributed as a chi-square (χ^2) with the test of df equal to the difference in the number of parameters estimated in each model (Neale & Cardon, 1992). The best-fitting model was selected based on a change in χ^2 not representing a significant deterioration in fit. If the χ^2 is not significant, the model with the fewer parameters is preferred for being more parsimonious. The likelihood ratio χ^2 test can only be employed to assess nested models (sub-models of the full model). Nested models can drop or fix one or more paths, and therefore test the fit of more parsimonious models. For non-nested models, the Akaike's information criteria (AIC) (computed as $\chi^2 - 2df$) was employed to compare the fit of alternative models. Lower AIC values indicate less difference between the observed and predicted covariance, and therefore reflect a better fit (Williams & Holahan, 1994). An AIC difference between models of less than 2 provides substantial evidence for both models; a difference between 3 and 7 indicates considerably less support for the model with the higher AIC; lastly, a difference greater than 10 suggests that the higher AIC model is very unlikely (Wagenmakers & Farrell, 2004).

Information about the accuracy and significance of parameter estimates was obtained by likelihood-based confidence intervals (CIs) (straddling zero indicative of non-significance). In this method a parameter is progressively moved away from its maximum likelihood estimate in either direction (while the other model parameters are optimised) until the difference in fit, distributed as a chi-square with one degree of freedom, is significant (for a change in df of 1, the statistically significant change is 3.84) (Neale & Miller, 1997).

3.3.3.2 Overview of the twin method

Based on the different genetic relatedness between twin pairs (MZ twins share 100% of their genes, whereas DZ twins share, on average, 50% of their segregating genes), and the assumption that shared environmental influences are expected to correlate to the same extent regardless of zygosity, phenotypic variance for a single trait (univariate analyses) can be dissected into additive genetic (A), dominant genetic (D) or shared environmental (C), and non-shared environmental (E) components (which also subsumes measurement error) (Plomin et al., 2008; Rijdsdijk & Sham, 2002). In brief, when similarity of MZ twins is greater than DZ twins, a genetic contribution to trait variation is implicated. If trait variation was solely influenced by genetic effects, then MZ similarity should be twice as great as similarity between DZ pairs. If not, this indicates that environmental influences that twin pairs share in common have contributed to greater similarity. Finally, if MZ twins, despite sharing all their genes do not fully correlate, this implicates that environmental factors unique to each twin have decreased phenotypic similarity.

3.3.3.3 Univariate genetic analyses

Univariate genetic models were fitted to data to inform parameter selection for multivariate models. These use twin correlations to decompose variance into the parameters A, C or D, and E (see section 1.3.1 for more details). Because C and D cannot be modelled simultaneously in the classical twin model (Rijdsdijk & Sham, 2002), the choice of whether to fit C or D was based on twin correlations. If MZ correlations were more than double DZ correlations, an ADE model was fitted. In the presence of additional significant variance differences by zygosity, rater contrast effects are modelled. For traits where DZ correlations were around half MZ correlations an ACE model was fitted.

Within the univariate modelling the presence of sex-specific influences on the phenotypes was tested. Models were fitted to test whether the magnitude of aetiological factors influencing males and females differ (quantitative sex differences), whether the aetiological factors influencing males differ to those influencing females (qualitative sex differences), and whether there are phenotypic variance differences between males and females (scalar sex differences).

Aetiological and variance sex differences are tested by employing a series of nested models with different constraints. In the full sex limitation model, all sex differences are allowed. However, we cannot allow for both qualitative genetic and shared environmental sex differences at the same time in samples that only have twin pairs reared together. Accordingly we first run a *full sex limitation* model that allows for a different genetic correlation between males and females in DZ opposite-sex (OS) pairs (but where the shared environmental correlation is fixed to 1.00 (in line with DZ same-sex (SS) twin pairs)). Next, we run a full sex limitation model that allows for a different shared environmental correlation between males and females in DZOS pairs (but where the genetic correlation is fixed to 0.5 (in line with DZ same-sex (SS) twin pairs)). Qualitative sex differences can be tested by comparing DZSS twins and DZOS twins; to the extent that DZSS twins are more similar than DZOS twins, qualitatively different aetiological influences for males and females are implied. Significant qualitative genetic sex differences are indicated by a genetic correlation between DZOS pairs less than 0.5, and significant qualitative environmental sex differences are indicated by a shared environmental correlation between DZOS pairs less than 1.00.

Then, we run the *common effects model*, which drops all qualitative sex differences (by constraining the genetic and shared environmental correlation for DZOS pairs to equal that for DZSS twin pairs). If this model does not represent a significant drop in fit we know that the same genes and the same environments affect the population variance in a trait for females and males.

Next, we run a *scalar model*, which does not allow for qualitative or quantitative sex differences (by further constraining aetiological parameters to be equal in males and females). If this model does not represent a significant drop in fit compared to the common effects model, then the magnitude of aetiological influences is the same across males and females.

In the final *null model*, no sex differences are modelled (no qualitative or quantitative sex differences, and we additionally constrain phenotypic variances to be equal across males and females). If this model does not represent a significant deterioration in fit compared to the scalar model, then there are no sex differences (a significant drop in fit implicates scalar (variance differences)).

3.3.3.4 Parameter selection for multivariate models informed by univariate genetic analyses

For the cognitive measures the DZ correlations (r_{DZ}) were around or more than half of the MZ correlations (r_{MZ}), leading us to fit an ACE model (as opposed to D). For the ADHD ratings r_{MZ} were more than twice the r_{DZ} . In the absence of significant MZ/DZ variance differences ($p > 0.01$), we fitted an ADE model to behavioural ratings. Using a p-value threshold of 0.01, there were neither qualitative nor quantitative sex differences, although gender-specific phenotypic variance differences were observed for the majority of the traits. Due to the lack of sex differences in the univariate analyses beyond scalar differences, the computational intensity of modelling sex effects and additional power issues (Neale, Roysamb, & Jacobson, 2006), only scalar differences between males and females were allowed in the multivariate models (male phenotypic variances were pre- and post-multiplied by a scaling factor).

3.3.3.5 Multivariate genetic analyses

Multivariate designs offer greater power by decreasing the rate of false positive (type I) error rates (by reducing multiple testing), and taking into account the covariance among phenotypes for each individual. Accordingly, as multivariate models have improved power over univariate models (Schmitz et al., 1998), only multivariate parameter estimates are presented. Multivariate studies are also able to partition phenotypic variance of individual traits, and the covariance between two or more traits. Within-twin cross-trait (WTCT) (phenotypic) correlations (e.g. hyperactivity for twin 1 and inattention for twin 1), reflect the degree to which two traits covary. Multivariate genetic analyses use the power given by the MZ:DZ ratio of cross-twin cross-trait (CTCT) correlations (e.g. inattention for twin 1 and hyperactivity-impulsivity for twin 2) to decompose the covariation between traits into A, C/D and E influences, utilising the same logic as univariate genetic analyses (Rijsdijk & Sham, 2002) (see section 1.3.1.3).

Saturated phenotypic model: The saturated model fully describes all the data, modelling the observed means and variances without dissecting variance or covariance into aetiological components. This model uses the maximum number of free parameters and provides a baseline comparison for subsequent genetic models. A Gaussian decomposition is fit to the data, which allowed us to test the assumptions of the twin model: no mean or variance differences within traits across twin 1 and twin 2, and across MZ and DZ twin pairs. These assumptions were all met ($p > 0.01$). In addition, phenotypic correlations across traits were equated across twins in a pair and zygosity groups to obtain phenotypic correlations representative of the whole sample. Table 3.2 presents maximum-likelihood correlations from data analysis, which includes these assumptions.

Correlated factors solution of the full Cholesky Decomposition: A triangular decomposition was run and converted to the mathematical equivalent correlated factors solution (Loehlin, 1996) (see Figure 3.1), in which the order of traits is arbitrary. The mathematical solution allows the estimation of the extent to which the same genetic or environmental factors contribute to trait covariation (see 1.3.1.5 for more details). Aetiological correlations provide an estimate of the degree of overlapping aetiological factors between two traits and vary from 0 (indicative of no overlap) to 1 (reflecting complete overlap).

Three-variable Cholesky Decomposition: In the Cholesky, a triangular decomposition is used, to decompose the variance in each phenotype and covariance between the phenotypes into aetiological influences. The Cholesky Decomposition (see Figure 3.2) partitions variance into shared and specific influences. The first set of latent factors can influence all traits; the second set of latent factors can influence trait 2 and trait 3 (and are independent of influences shared with trait 1). The third set of latent factors is unique to trait 3. As such this procedure is similar to hierarchical regression analyses, where the independent contribution of a predictor variable is tested, after controlling for shared variance with other predictor variables (Haworth, Kovas, Dale, & Plomin, 2008).

Although the ordering of variables in the Cholesky Decomposition may be arbitrary, the order of the traits in our analyses was decided a priori, with a view to estimating the aetiological influences that contribute to the covariance between inattention symptoms and cognitive

factors, independent of influences underlying hyperactivity-impulsivity. Accordingly, hyperactivity-impulsivity was assigned as the first measured variable; as such for these analyses we present the triangular decomposition.

3.4 Results

Means and standard deviations for measures included in this study are given in Table 3.1. Given the variance differences between genders, means and standard deviations are presented separately for males and females.

Maximum-likelihood twin pair correlations are provided in Table 3.2. Due to the lack of quantitative or qualitative sex differences, MZ and DZ twin correlations are not presented separately for each sex.

Table 3.1 Means and standard deviations

	Hyperactivity- impulsivity ^a	Inattention ^a	MRT ^b	RTV ^b	CE ^c
MZM	11.06 (8.61)	12.70 (8.95)	1481.79 (322.16)	619.06 (350.81)	116.52 (34.29)
	N=222	n=222	n=237	n=237	n=243
MZF	6.74 (5.89)	7.79 (6.51)	1587.52 (310.24)	629.94 (364.15)	96.42 (31.47)
	N=234	n=234	n=257	n=257	n=267
<i>DZM</i>	<i>11.53 (9.64)</i>	<i>14.25 (11.14)</i>	<i>1497.49 (322.12)</i>	<i>631.01 (376.52)</i>	<i>115.59 (32.90)</i>
	<i>N=360</i>	<i>n=360</i>	<i>n=376</i>	<i>n=376</i>	<i>n=391</i>
<i>DZF</i>	<i>7.32 (6.49)</i>	<i>9.06 (7.88)</i>	<i>1551.48 (314.56)</i>	<i>628.04 (359.12)</i>	<i>95.61 (33.14)</i>
	<i>N=343</i>	<i>n=343</i>	<i>n=377</i>	<i>n=377</i>	<i>n=389</i>

Note: Abbreviations- MRT: mean reaction time; RTV; Reaction time variability; CE: commission errors; MZM: monozygotic males; MZF: monozygotic females; DZM: dizygotic males; DZF: dizygotic females; ^a Sum of parent and teacher ratings; ^b Sum of unstandardised scores across baseline condition of the fast task and slow condition of the go/no-go (GNG) task; ^c Sum of percentage of CE across slow and fast conditions of the GNG task; n: number of observations; **MZ data in bold typeface**, *DZ data in italic typeface*

Table 3.2 Twin pair correlations (and 95% confidence intervals) ^a

	Hyperactivity- impulsivity ^b	Inattention ^b	MRT ^c	RTV ^c	CE ^d
Twin correlations					
MZ / DZ	Twin 1				
	Twin 2				
Hyperactivity-impulsivity ^b	0.73 (0.66/0.76) <i>0.30 (0.20/0.32)</i>	<i>0.16 (0.08/0.24)</i>	<i>0.01 (-0.06/0.09)</i>	<i>0.04 (-0.03/0.08)</i>	<i>0.05 (-0.03/0.09)</i>
Inattention ^b	0.45 (0.38/0.54)	0.62 (0.53/0.68) <i>0.08 (0.02/0.15)</i>	<i>0.01 (-0.06/0.03)</i>	<i>0.03 (-0.04/0.09)</i>	<i>0.01 (-0.06/0.08)</i>
MRT ^c	0.09 (0.02/0.19)	0.19 (0.12/0.29)	0.60 (0.51/0.73) <i>0.33 (0.23/0.33)</i>	<i>0.23 (0.14/0.26)</i>	<i>0.01 (-0.06/0.08)</i>
RTV ^c	0.13 (0.06/0.14)	0.18 (0.11/0.21)	0.44 (0.36/0.46)	0.44 (0.34/0.48) <i>0.22 (0.12/0.27)</i>	<i>0.06 (-0.02/0.06)</i>
CE ^d	0.05 (-0.03/0.12)	0.04 (-0.05/0.11)	-0.09 (-0.16/-0.08)	0.02 (-0.06/0.04)	0.28 (0.17/0.39) <i>0.14 (0.03/0.23)</i>

Note: Abbreviations- MRT: Mean reaction time; RTV; Reaction time variability; CE: Commission errors; ^a Estimated using maximum likelihood estimation; ^b Sum of parent and teacher ratings; ^c Sum of unstandardised data scores across baseline condition of the fast task and slow condition of the go/no-go (GNG) task; ^d Sum of percentage of CE across slow and fast conditions of the GNG task; **MZ data in bold typeface, DZ data in italic typeface**

3.4.1 Phenotypic and aetiological association between cognitive impairments and the two symptom domains of ADHD considered separately

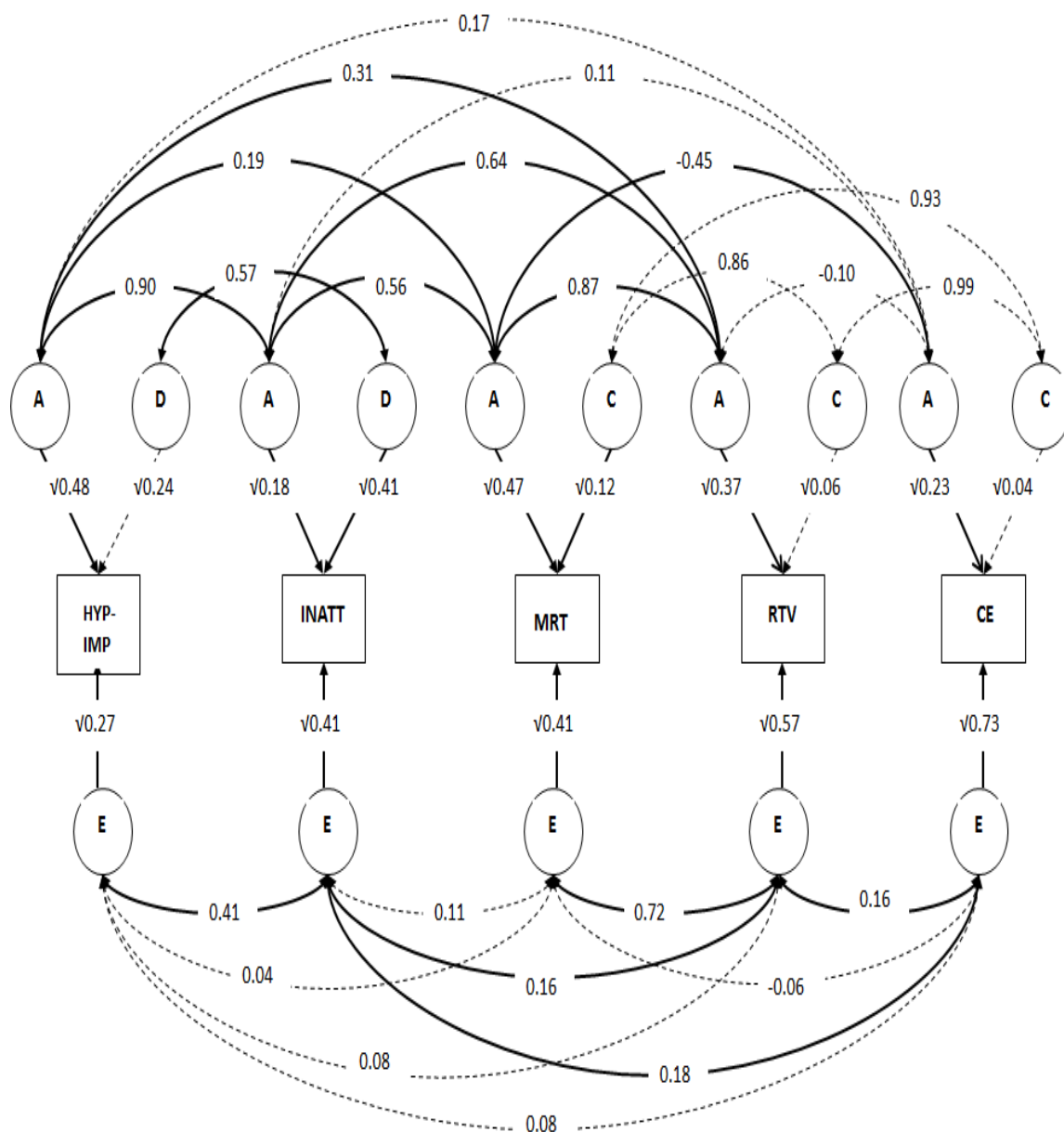
Parameter estimates are presented in Table 3.3 and Figure 3.1 from the full correlated factors solution of the Cholesky Decomposition. To avoid artificially inflating parameters, estimates from the full model are provided, and non-significance is indicated by confidence intervals that include zero.

Phenotypic correlations for either behavioural rating were stronger with RTV (0.16 to 0.24) versus CE (0.09 to 0.12). The greatest phenotypic association was observed between RTV and inattention (0.24), which was significantly larger than the phenotypic covariation observed between CE and inattention (0.12).

Genetic correlations between symptom domains and cognitive variables refer to additive genetic effects, as dominant genetic effects do not contribute to the covariation between these phenotypes. However, these additive genetic correlations are referred to below and throughout this thesis more broadly as genetic influences. Genetic correlations in particular indicated a different pattern of association with the two ADHD symptoms for RT variables versus CE, with the strongest genetic association observed between RTV and inattention ($r_G = 0.64$). A moderate genetic association was also observed between RTV and hyperactivity-impulsivity symptoms ($r_G = 0.31$). In contrast, we found lower genetic correlations for CE, although there was less differentiation with symptom domains, with genetic correlations of 0.11 and 0.17 for inattention and hyperactivity-impulsivity, respectively.

The vast majority (68% to 87%) of the phenotypic covariance between RT-related factors and either ADHD behavioural dimension was due to shared genetic (additive) effects. A greater degree of differentiation was observed when partitioning the contribution of shared genetic factors to the phenotypic covariation of CE for ADHD symptom domains (inattention (19%) and hyperactivity-impulsivity (61%)).

Figure 3.1 Correlated factors solution of the full Cholesky Decomposition



Note: Significant parameters are indicated with solid lines; non-significant parameters in dotted lines; Abbreviations- HYP-IMP: Hyperactivity-impulsivity; INATT: Inattention; MRT: Mean reaction time; RTV: Reaction time variability; CE: Commission errors; Model presented for one twin only for ease of presentation;

Table 3.3 Phenotypic correlations and standardised parameter estimates (with 95% confidence intervals (CIs)) from the correlated factors solution of the full Cholesky Decomposition, within and across ADHD behavioural ratings and cognitive measures

	Hyperactivity- Impulsivity^a	Inattention^a	MRT^b	RTV^b	CE^c
Phenotypic correlations					
Inattention ^a	0.58 (0.54/0.62)	-	-	-	-
MRT ^b	0.10 (0.04/0.17)	0.21 (0.15/0.27)	-	-	-
RTV ^b	0.16 (0.10/0.22)	0.24 (0.18/0.30)	0.79 (0.76/0.81)	-	-
CE ^c	0.09 (0.03/0.15)	0.12 (0.06/0.17)	-0.11 (-0.17/-0.05)	0.12 (0.07/0.18)	-
Additive genetic influences					
Hyperactivity-impulsivity ^a	0.48 (0.10/0.76)	0.27 (46%)	0.09 (87%)	0.13 (81%)	0.06 (61%)
Inattention ^a	0.90 (0.39/0.99)	0.18 (0.05/0.40)	0.16 (78%)	0.17 (68%)	0.02 (19%)
MRT ^b	0.19 (0.03/0.47)	0.56 (0.29/0.94)	0.47 (0.28/0.62)	0.36 (46%)	-0.15 *
RTV ^b	0.31 (0.13/0.72)	0.64 (0.33/1.00)	0.87 (0.72/1.00)	0.37 (0.15/0.51)	-0.03 *
CE ^c	0.17 (-0.06/0.57)	0.11 (-0.38/0.49)	-0.45 (-0.96/-0.03)	-0.10 (-0.91/0.36)	0.23 (0.03/0.36)
Dominant genetic influences (Hyperactivity-impulsivity, inattention) / Common environmental influences (MRT, RTV, CE)					
Hyperactivity-impulsivity ^a	0.24 (0.00/0.63)	0.18 (31%)	-	-	-

Inattention ^a	0.57 (0.47/1.00)	0.41 (0.17/0.57)	-	-	-
MRT ^b	-	-	0.12 (0.01/0.27)	0.07 (9%)	0.06 *
RTV ^b	-	-	0.86 (-1.00/1.00)	0.06 (0.00/0.23)	0.05 *
CE ^c	-	-	0.93 (-1.00/1.00)	0.99 (-1.00/1.00)	0.04 (0.00/0.20)

Individual-specific environmental influences

Hyperactivity-impulsivity ^a	0.27 (0.22/0.34)	0.14 (24%)	0.01 (13%)	0.03 (19%)	0.04 (39%)
Inattention ^a	0.41 (0.28/0.52)	0.41 (0.33/0.51)	0.05 (22%)	0.08 (32%)	0.10 (82%)
MRT ^b	0.04 (-0.09/0.17)	0.11 (-0.01/0.24)	0.41 (0.34/0.49)	0.35 (45%)	-0.03 *
RTV ^b	0.08 (-0.04/0.20)	0.16 (0.05/0.27)	0.72 (0.66/0.77)	0.57 (0.48/0.67)	0.10 *
CE ^c	0.08 (-0.04/0.20)	0.18 (0.07/0.29)	-0.06 (-0.17/0.05)	0.16 (0.05/0.27)	0.73 (0.63/0.83)

Note: In the upper part of the table, the phenotypic correlations are given. In the next quarter, additive genetic estimates (with 95% CIs) of each variable given on the diagonal. The additive genetic correlations between variables (with 95% CIs), and the contribution of additive genetic factors to the phenotypic correlation between variables (with the percentage in brackets), are given above and below the diagonal. The same information is presented for dominant genetic/shared environmental and individual-specific environmental influences in the third and lower quarters of the table, respectively. Significant parameters indicated by bold typeface; MRT: Mean reaction time; RTV: Reaction time variability; CE: Commission errors; ^a Sum of parent and teacher ratings; ^b Sum of unstandardised scores across baseline condition of the fast task and slow condition of the go/no-go (GNG) task; ^c Sum of percentage of CE across the GNG task; * It was not possible to formally estimate the proportional contribution to phenotypic covariation, due to the presence of both positive and negative aetiological correlations;

3.4.2 Aetiological association between RTV and inattention, independent of hyperactivity-impulsivity

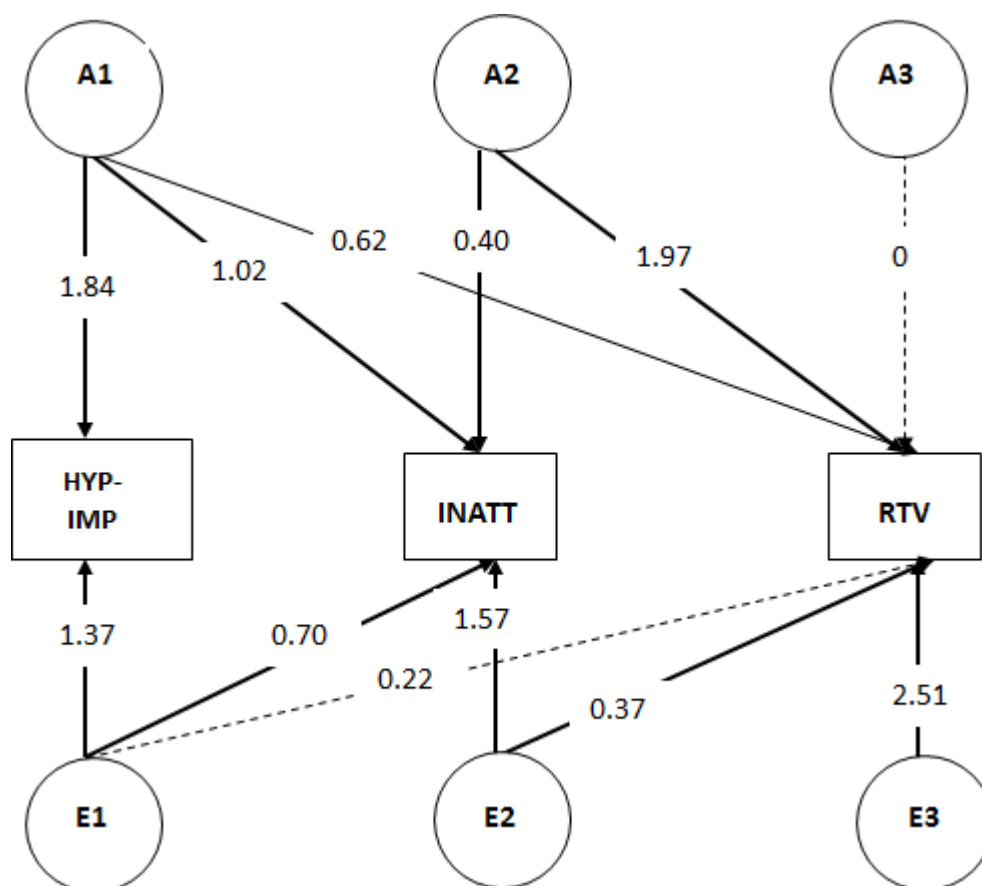
Given that the strongest genetic correlation between symptom scores and a cognitive variable emerged between inattention and RTV, this was investigated further in the Cholesky Decomposition. Specifically we wanted to test with the Cholesky Decomposition, how much of the aetiological association between RTV and inattention was independent of hyperactivity-impulsivity. This can be estimated by summing the product of Cholesky additive genetic/individual-specific environmental paths that are not shared with hyperactivity-impulsivity, and taking them as a percentage of the total additive genetic/individual-specific environmental covariance between inattention and RTV data (C and D do not underlie both inattention and RTV and so do not contribute to the covariation between these two traits).

Using the parameter estimates from the Cholesky Decomposition (Figure 3.2), we estimated that 55% of the genetic covariance between inattention and RTV occurred independently of genetic effects underlying hyperactivity-impulsivity:

$$((0.40 \times 1.97) / ((0.40 \times 1.97) + (1.02 \times 0.62)) = 0.79 / (0.79 + 0.63) = 0.79 / 1.42 = 0.55).$$

In a similar vein, 79% of the individual-specific environmental covariance between RTV and inattention was independent of E underlying hyperactivity-impulsivity.

Figure 3.2 Additive genetic and individual-specific environmental parameter estimates from the three-variable Cholesky Decomposition



Note: unstandardised parameter estimates; significant parameters are indicated with solid lines; non-significant parameters with dotted lines; Abbreviations- HYP-IMP: Hyperactivity-impulsivity; INATT: Inattention; RTV: Reaction time variability; Model presented for one twin only for ease of presentation and for additive genetic (A) and individual-specific environmental (E) influences only (the only aetiological factors that contributed to covariation between either hyperactivity-impulsivity and inattention with RTV).

3.5 Discussion

We investigated the genetic associations of the two ADHD symptom domains of inattention and hyperactivity-impulsivity with key cognitive impairments known to be associated with the familial risk for ADHD. Multivariate twin model fitting identified two cognitive processes phenotypically associated with ADHD symptoms, captured by RTV and CE, which showed different genetic relationships to the two ADHD symptom domains. The findings are consistent with our previous report on two familial cognitive impairment factors in ADHD (Kuntsi et al., 2010), but further extend the previous observations by investigating the two ADHD symptom dimensions separately and by using a twin design that can distinguish between genetic and shared environmental effects that underlie familial influences.

Our previous analyses on a large ADHD and control sibling-pair sample indicated that RT measures index a large familial cognitive impairment factor in ADHD that accounts for 85% of the familial influences on ADHD (Kuntsi et al., 2010). Here we show, with a large population-based twin sample, that the RTV-ADHD association reflects largely genetic influences that RTV shares with inattention ($r_G = 0.64$). Although the strongest genetic association was observed between RTV and inattention, a moderate genetic association was also observed between RTV and hyperactive-impulsive symptoms ($r_G = 0.31$). However, our further analyses showed that just over half (55%) of the genetic covariance between RTV and inattention was independent of genetic influences on hyperactivity-impulsivity. This degree of separation is notable, given the observed strong additive genetic correlation between inattention and hyperactivity symptoms ($r_A = 0.90$), in line with previous findings (Greven, Asherson et al., 2011; McLoughlin et al., 2011; McLoughlin et al., 2007; Paloyelis et al., 2010). Our findings also confirm the previous observation that mean reaction time (MRT) indexes largely the same genetic liability as RTV (Wood, Asherson et al., 2010), observed in the high genetic correlation of 0.87.

The second, smaller familial cognitive impairment factor in ADHD in our previous analyses captured CE as well as omission errors, and accounted for 13% of the familial influences on ADHD (Kuntsi et al., 2010). However, in contrast to the stronger aetiological association between RTV and inattention, the current results suggest that the CE-ADHD association reflects influences CE shares with both inattention and hyperactivity-impulsivity, although the genetic correlations were overall low ($r_G = 0.11$ and 0.17 , respectively) and non-significant. Further twin

studies are required to clarify whether the low genetic correlations between CE and the ADHD symptom domains would emerge as significant in larger samples, although we note the consistency between the current and previous findings in the degree of genetic/familial association between CE and ADHD symptoms (Kuntsi et al., 2010).

Finally, the current findings clearly demonstrate the familial separation between the two indices of cognitive impairments, since there were no significant shared genetic or environmental (C) influences across RTV and CE. This is consistent with the familial separation that was identified in the previous study using children and adolescents with ADHD-C subtype, their siblings and control sibling pairs.

Our findings converge with previous studies using clinical phenotypes in highlighting the importance of both shared and unique aetiological pathways on the two symptom domains of ADHD. A recent analysis comparing factor models of ADHD symptoms in adolescents, found that a general combined factor with separable inattention and hyperactivity-impulsivity dimensions best explained the symptom data (Toplak et al., 2009); a pattern of findings that is reflected in the shared and unique genetic effects that influence inattention and hyperactivity-impulsivity. Here we demonstrate the degree of specificity that the cognitive impairment factors have in their genetic association with inattention and hyperactivity-impulsivity symptoms. The two cognitive impairments in ADHD may also interplay throughout development, leading to different outcomes for ADHD as individuals pass from childhood into adulthood (Halperin & Schulz, 2006; Halperin et al., 2008). Within such a developmental model (see section 1.5.3), the finding that RTV, reflecting an early-onset enduring deficit (Halperin & Schulz, 2006; Halperin et al., 2008), is associated specifically with inattention, may explain the developmental persistence of the inattentive symptom domain (Biederman et al., 2000; Larsson et al., 2006; Todd et al., 2008). The possible role of the cognitive processes described here in mediating genetic effects underlying the ADHD symptom domains is investigated in chapter 4. The potential of these cognitive processes contributing to the association of the two ADHD symptom domains with different patterns of comorbidity is an important direction for future research that arises from these findings, and is investigated in chapter 5.

A limitation of this study is that teacher ratings were missing for 151 individuals. Strengths of the study include the use of a population sampling strategy that is free from potential referral effects, which might bias estimates of the aetiological associations between co-occurring behavioural and cognitive phenotypes. We adopted a quantitative approach to the analysis of ADHD symptoms, which reflects the continuous nature of ADHD symptoms in the population (see section 1.3.2). The similarity between the findings presented here and the previous study using clinical cases of ADHD provide further evidence that ADHD reflects the extreme and impairing tail of quantitative traits for inattention and hyperactivity-impulsivity (Chen et al., 2008). This has implications for our understanding of the nature of ADHD by demonstrating the quantitative nature of ADHD at both the behavioural, cognitive and aetiological level. This further emphasises the importance of linking symptoms to impairments when defining the clinical condition, in line with suggestions from the National Institute for Clinical Health and Excellence (NICE, 2008) and supports the further use of population sampling strategies for investigating the separate neurobiological processes that underlie the clinical condition.

CHAPTER 4 QTL ANALYSIS OF PUTATIVE ADHD RISK MARKERS USING QUANTITATIVE BEHAVIOURAL MEASURES OF ADHD SYMPTOMS AND COGNITIVE IMPAIRMENTS

4.1 Abstract

In the previous chapter, cognitive impairment factors (reaction time variability (RTV) and commission errors (CE)) associated with attention-deficit/hyperactivity disorder (ADHD) displayed different genetic relationships with the two ADHD symptom domains when considered separately. RTV showed substantial genetic overlap with inattention compared to hyperactivity-impulsivity; whilst CE showed low genetic correlations with both hyperactivity-impulsivity and inattention. However what remains poorly understood is whether these distinct cognitive performance measures represent pleiotropic genetic effects (alternative manifestations of the same underlying genetic factors) or causal processes that mediate genetic effects on ADHD symptoms. In this study we test for molecular genetic associations between previously reported ADHD risk genetic variants and quantitative measures of ADHD symptoms and cognitive impairment factors using the quantitative trait loci (QTL) approach, in two independent samples. The first sample was the same population-based twin sample used in chapter 3. The second sample was a large international collaborative sample of ADHD-proband sibling pairs. The objective of this study was not to detect novel ADHD QTL, but to investigate putative ADHD genetic risk markers, and determine whether associations with ADHD as a clinical disorder can be confirmed with quantitative ADHD-related measures across both samples. In addition, we aimed to investigate whether overlapping genetic associations between cognitive and clinical measures represent pleiotropic or mediating effects. The overall design was to screen the population-based sample for associations, and where these were identified to use the clinical sample for replication. Analysis on the population-based sample yielded a number of nominally significant associations, although none withstood correction for multiple testing. These preliminary findings suggested that the norepinephrine transporter gene (SLC6A2) might be a potential genetic marker contributing to the association between CE and both ADHD behavioural ratings; the lack of any mediating effects suggest that these associations are more likely to reflect pleiotropic genetic effects. The serotonin receptor gene (5HT2A) was also identified as a potential risk factor, contributing to the observed association between RTV and inattention; in this case mediation analysis found that RTV mediated a substantial proportion of the association between 5HT2A and inattention. However, a detailed

analysis of SNPs spanning SLC6A2 and 5HT2A in a clinical sample using similar quantitative assessments of ADHD and cognitive performance measures, failed to replicate the associations seen in the population sample. Taken together, the results of this investigation are not convincing due to the lack of replication between the population-based and clinical samples. However, the finding for 5HT2A in the population samples remains of potential interest because the mediation analysis suggested that there might be a potential aetiological pathway from 5HT2A via RTV to inattention; consistent with the findings reported from the quantitative genetic analyses in chapter 3. Further studies are therefore required to confirm or refute this finding and extend to other genes that might act together with 5HT2A in a multi-gene pathway.

4.2 Introduction

In the past decade there have been considerable strides in identifying attention-deficit/hyperactivity disorder (ADHD) endophenotypes, objectively measured traits related to ADHD, which Gottesman & Gould (2003) argued theoretically should be less genetically complex than the disorder and lie closer to genetic liability and accordingly facilitate gene hunting efforts. However, there is a paucity of molecular genetic studies that have targeted ADHD-related quantitative measures as opposed to clinical ADHD (Zhou, Asherson et al., 2008).

The concept of an endophenotype was further elaborated by Kendler & Neale (2010). They argued that endophenotypes can be distinguished as either reflecting risk indicators or intermediate phenotypes (see section 1.6) (Kendler & Neale, 2010). Both types share genetic risk factors with clinical phenotypes. However, risk indicators covary with the disorder and represent pleiotropic genetic effects (multiple alternative manifestations of common genetic risk factors). Intermediate phenotypes are hypothesised to lie along the pathway from gene to clinical phenotype and represent causal processes that mediate genetic effects. Thus, identifying intermediate phenotypes is a promising approach to delineate pathophysiological processes underlying disorder presentation. Accordingly, elucidating processes underlying ADHD is one of the main attractions of intermediate phenotypes as opposed to facilitating molecular genetic investigations (Kuntsi, Neale et al., 2006; Meyer-Lindenberg, 2010). Increased understanding of the neurobiological underpinnings of ADHD may contribute to

facilitating intervention strategies, particularly by extending the study into animal models (Glahn & Blangero, 2011).

However, whether endophenotypes represent pleiotropic or mediating processes has largely been neglected (Kendler & Neale, 2010). The distinction of whether an endophenotype covaries with a disorder due to pleiotropy or mediation has been based on the assumption that some endophenotypes are more ‘cognitive’ (implicating mediating processes) rather than ‘clinical’ (implicating pleiotropic processes); but in the absence of formal testing it is not possible to distinguish between these effects (Asherson & Gurling, 2012). The clarification of which endophenotypes represent intermediate phenotypes that mediate aetiological effects on ADHD behaviours is an important step in order to identify neurobiological and developmental causal processes that might become the target of treatment interventions for ADHD (Kendler & Neale, 2010).

To date most progress in identifying common genetic variants associated with ADHD has come from candidate gene studies. Candidate gene association studies of clinical ADHD have yielded mixed findings, although several genetic variants have been replicated in two or more studies and therefore retain suggestive levels of evidence. Moreover, findings that remain significant after meta-analyses highlight the most promising genetic markers (Gizer et al., 2009) (see Table 1.2). However, much of the genetic variance of ADHD remains elusive (Kuntsi, Neale et al., 2006; Plomp et al., 2009; Purper-Ouakil et al., 2011).

There is substantial converging evidence from quantitative genetic analyses to corroborate a dimensional view of ADHD genetic liability (Chen et al., 2008) (see section 1.3.2). However, there is a paucity of molecular genetic studies that have targeted ADHD-related quantitative measures as opposed to clinical ADHD (Zhou, Asherson et al., 2008). A limitation of clinical studies is that they often are unable to look separately at the two symptom domains of ADHD. The alternative strategy of investigating genetic associations with quantitative ADHD trait ratings in line with the quantitative trait loci (QTL) approach, has only been used in a few studies, with no clear pattern of findings emerging (Bidwell et al., 2011; Curran et al., 2001; Ilott, Saudino, & Asherson, 2010; Ilott, Saudino, Wood et al., 2010; Lasky-Su, Lange et al., 2008; Mill et al., 2002; Mill et al., 2005; Waldman et al., 1998). (See section 1.4.1.3).

Reviews of studies of the molecular genetic correlates of cognitive endophenotypes in ADHD samples have concluded that individual studies have yielded similarly mixed findings (Bellgrove et al., 2008; Kebir & Joober, 2011; Kebir et al., 2009). A recent review of 47 candidate gene association studies targeting cognitive measures found that the majority ($n = 36$) investigated only one candidate gene, and that the majority have examined associations with only two usual suspects: the dopamine transporter (DAT1) and dopamine receptor D4 (DRD4) gene (Kebir & Joober, 2011). Overall, the strongest evidence is for increased reaction time variability (RTV) associated, although paradoxically, with the absence of the DRD4 7-repeat allele (a risk allele for ADHD) (Kebir et al., 2009) (see section 1.6.4.2).

Overall the evidence from QTL studies targeting quantitative measures of ADHD and putative ADHD cognitive markers is mixed. Meta-analyses of QTL studies are currently lacking, though this may result in the identification of some more consistently replicated and promising associations, with a potential obstacle being the wide range of ADHD behavioural rating scales and task paradigms employed (Kebir et al., 2009).

However, as already highlighted the utility of endophenotypes goes beyond providing an alternative target to clinically derived groups in molecular genetic investigations, and may clarify the risk pathways between genotypes and clinical phenotypes. As yet few studies have investigated the mediating role of endophenotypes in pathways from genes to ADHD symptoms. One study using a series of regression analyses examined 11 different measures of executive functioning, and found no evidence that they mediated the association between three single nucleotide polymorphisms (SNPs) in the adrenergic receptor α -2A (ADRA2A) gene and ADHD affection status (Waldman et al., 2006). In another study, low conscientiousness and high neuroticism were found to partially mediate the genetic association between a genetic composite (based on the number of risk alleles for DRD4, DAT1 and ADRA2A) and ADHD (particularly inattentive) symptoms (Martel, Nikolas, Jernigan, Friderici, & Nigg, 2010). In a large cohort sample of over 4000 children (Avon Longitudinal Study of Parents and Children), the association with the catechol-O-methyltransferase (COMT) gene and antisocial behaviour in ADHD was partially mediated by impaired social cognition, whereas impairments in executive control represented pleiotropic effects (Langley, Heron, O'Donovan, Owen, & Thapar, 2010).

Overall, our knowledge of the causal links from risk genes to ADHD remains very limited, particularly so for the two ADHD dimensions considered separately. In chapter 3 we identified that two key familial cognitive impairments phenotypically associated with ADHD, captured by reaction time variability (RTV) and commission errors (CE), showed different genetic relationships to the two ADHD symptom domains. These findings converge with previous studies using clinical phenotypes in highlighting the importance of both shared and unique aetiological pathways on the two symptom domains of ADHD. However, we do not yet know whether they reflect causal processes that mediate genetic risks on ADHD or pleiotropic genetic effects.

In the present study we extend our previous research of investigating and refining quantitative measures of ADHD for molecular genetic studies. Specifically we aimed to investigate associations with previously implicated ADHD risk genes across ADHD behavioural ratings and objectively-measured ADHD-related traits, utilising a QTL approach in a population-based twin sample. Employing an unselected, general population sample overcomes potential biases inherent in clinical samples and allows us to extrapolate findings to the general population. This also will allow us to separately examine the molecular genetic correlates of the two behavioural dimensions of ADHD, and assess the suitability of our selected cognitive intermediate ADHD phenotypes as tapping into the same genetic factors underlying ADHD as a disorder. Overlapping genetic associations across both cognitive and behavioural measures are of particular interest, as our second aim was to test where relevant, for the potentially mediating role of cognitive measures in the association between specific genetic variants and ADHD symptoms. The third aim was to carry out a detailed analysis of SNPs spanning the length of risk genes that showed overlapping associations in our population-based analysis, in a large sample of ADHD-proband sibling-pairs. This attempt at replication, using identical quantitative trait measures as those employed in our initial population-based sample, allows us to test the comparability of findings across samples.

PART A: A GENERAL POPULATION TWIN SAMPLE

4.3 Methodology

4.3.1 Sample and procedure

Participants are members of the Study of Activity and Impulsivity Levels in children (SAIL), a general population sample of twins aged 7 to 10 years (see section 3.3.1 for more details).

4.3.2 Measures

IQ was assessed using four subtests from the Wechsler Intelligence Scales for Children (Third Edition) (Wechsler, 1991) (see section 3.3.2.2). ADHD behavioural ratings are described in section 3.3.2.1. Cognitive data was obtained using the go/no-go (GNG) task (Borger & van der Meere, 2000; Kuntsi, Andreou et al., 2005; van der Meere et al., 1995) and the fast task (Andreou et al., 2007; Kuntsi, Andreou et al., 2005; Kuntsi, Rogers et al., 2006); (see section 3.3.2.3 and 3.3.2.4 for more details of specific tasks and section 3.3.2.5 for details on the selection and treatment of cognitive variables for analysis).

4.3.3 Selection of variables to take forward for molecular genetic analyses

The selection of which variables to target in molecular genetic studies is an important consideration, as correcting for multiple testing contributes to decreased statistical power.

Accordingly the selection of variables to target in this study was informed by the corresponding quantitative genetic analyses (see chapter 3). Moreover, we set out to identify if the pattern of genetic associations were in line with the findings that emerged from the quantitative genetic analysis. We therefore expected to find: overlapping associations for both ADHD dimensions and reaction time (RT) measures and no overlapping associations between RTV and CE. Although the genetic correlations between CE and both ADHD behavioural dimensions were non-significant in our previous analysis, we speculate that this may be due to the sample size, and so a potential question to address was whether significant overlapping genetic associations across these variables of interest would emerge using molecular genetic analyses.

The high genetic correlation of 0.83 across mean RT (MRT) and RTV indicate that they index largely the same genetic liability, with RTV further showing higher phenotypic and genetic

association with ADHD symptom ratings, in line with previous findings (Kuntsi et al., 2010; Wood, Asherson et al., 2010). Therefore, in order to reduce multiple testing, MRT was not taken forward for analyses. Variables included in the molecular genetic analysis were inattention ratings, hyperactivity-impulsivity ratings, RTV scores and percentage of CE.

Tests of allelic association were performed on the exact same final variables as that used in corresponding quantitative genetic analysis (chapter 3). Therefore all measures were regressed for age and sex (and cognitive variables were additionally regressed for IQ (see 3.3.2.5)), and then transformed (with the exception of CE) using the optimised minimal skew 'lnskew0' command in STATA (Stata, 2005).

4.3.4 Genotyping

19 Polymorphisms were selected on the basis of previous reports of association with ADHD (see Table B.1 in Appendix B). SNPs were included from the following genes: cadherin 13 (CDH13), ciliary neurotrophic factor receptor (CNTFR), DAT1, DRD4, serotonin receptor 1B (HTR1B), monoamine oxidase A (MAOA), norepinephrine transporter (SLC6A2/NET1), synaptosomal-associated protein 25 (SNAP-25), tryptophan hydroxylase 2 (TPH2), and the serotonin receptor (5HT2A). Variable Number Tandem Repeat (VNTR) polymorphisms nominated included: COMT Val158Met, DAT1 3'UTR, DAT1 intron 8, DRD4 exon 3, and 5-HTTLPR.

DNA was extracted from buccal swabs (as described elsewhere (Freeman et al., 2003)). SNPs were genotyped using the Sequenom system. VNTR polymorphisms were genotyped manually using agarose gel electrophoresis (Asherson et al., 2007; Brookes, Xu, Chen, Zhou, Neale, Lowe, Anney et al., 2006; Xu, Mill et al., 2005). 13 children did not participate in genotyping.

Genotyping errors were estimated from genotype discordance rates within MZ twin pairs using PEDSTATS, a feature of the Quantitative Transmission Disequilibrium Test (QTDT) program (Abecasis, Cardon, & Cookson, 2000). DAT1 SNPs rs40184 and rs2625211 were excluded due to a high rate of MZ discordance (error rates of 5.70% and 4.18%, respectively). For the remaining 17 markers, the average MZ discordance error rate was 1.72% (ranging from no error rates

found for two markers to 3.42%). All other MZ discordance errors were re-coded as missing. After these quality control steps, the 5-HTTLPR VNTR was omitted as it had a high level (10.4%) of missing data. All 16 markers used in the final analysis conformed to Hardy-Weinberg equilibrium ($p > 0.01$).

4.3.5 Analyses

4.3.5.1 Genetic association

Tests of allelic association were performed using the QTDT program (Abecasis et al., 2000). QTDT tests for association in a variance components framework. Three models of association were tested using a likelihood ratio test implemented in QTDT: the 'Total Association' test (AT), the 'Within-Test' of association (AW) and the test of 'Population Stratification' (AP). Overall association was tested using the AT model which assesses both the within-pair differences as well as between pair sums (i.e. the correlation between phenotypic and genotypic differences and sums for each twin pair) and is the most powerful test in the absence of stratification effects. In contrast, the AW assesses the within component only. The within-pair design of the AW means that it is unaffected by between-family stratification effects, yet is less powerful than the AT in the absence of stratification. Based on the differences between these two models, the significance of association should reflect stratification effects. To evaluate this we modelled association using the AP test, which compares the significance from the between component versus the within component of association. Stratification effects are dismissed when these components are equal and therefore $p \geq 0.05$. In this instance, results are interpreted from the AT. Conversely, results are interpreted from the AW if significant stratification effects are detected.

VNTR markers were tested using the 'multi-allelic' function in QTDT. This provides a single p-value for tests of alleles with an allele frequency > 0.05 .

UNPHASED (<http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/>) (Dudbridge, 2008), was used to test X-linked MAOA marker (rs6323) because QTDT cannot deal with such data. UNPHASED has no means for handling MZ twin data; therefore mean phenotypic scores

for MZ pairs were used in these analyses, and MZ pairs entered as singletons (approach recommended by Professor Pak Sham).

To correct for multiple testing a Bonferroni correction for the 16 markers analysed was applied, requiring $p \leq 0.003$ to attain study-wise statistical significance (although this does not account for the multiple phenotypes also targeted).

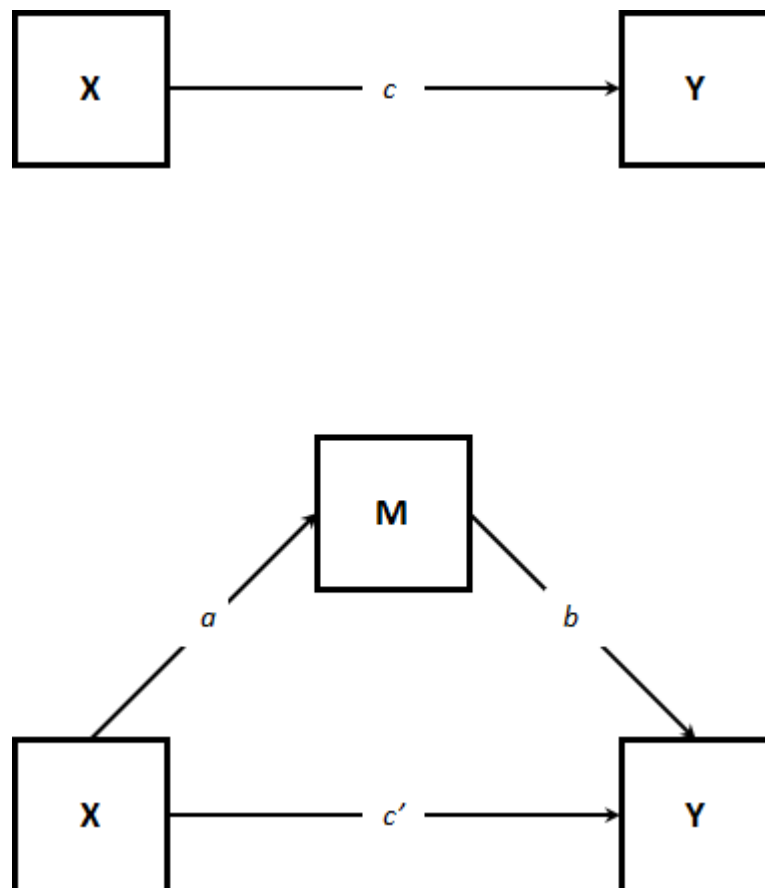
Genetic associations that overlapped across behavioural ratings and cognitive measures were identified and taken forward to test whether associations with cognitive measures reflected pleiotropic or mediating genetic effects.

4.3.5.2 Mediation tests

The mediation model is presented in Figure 4.1. The coefficient c' represents the direct effect of X (the independent variable (predictor (in our case genotype)) on Y (outcome (in our case behavioural rating)), with the effect of M (mediator (in our case cognitive measure)) removed. The coefficient a represents the effect of the SNP on the mediator, and the coefficient b represents the effect of the mediator on the outcome. Therefore the mediated (or indirect) effect of the SNP on the outcome via the mediator is represented as ab . The total effect of the SNP on the outcome (including the direct and indirect effect) is represented by c , and is estimated as $ab + c'$.

Approaches to test mediation have been subjected to extensive research over the last two decades (Fairchild & MacKinnon, 2009). (See MacKinnon, Lockwood, Hoffman, West, & Sheets, (2002) for review of 14 methods). In brief, the various methods can be categorised into three approaches. The causal steps method by Baron & Kenny (1986) ranks amongst one of the most frequently used approaches, but has several limitations, mainly that it specifies a series of regressions to test relationships between all variables to determine whether there is full, partial or no mediation. Accordingly this method does not quantify the mediated effect, and does not provide a test of whether the effect is significant. Moreover, the necessity for a significant total effect from dependent and independent variables as stated by the Baron & Kenny approach for mediation to occur, has been subsequently challenged.

Figure 4.1 Path diagram for a mediation model



Note: adapted from Fritz & MacKinnon (2008); Abbreviations- X: Independent variable; M: Mediating variable; Y: Dependent variable; The mediation model decomposes the total effect of X on Y (c), into two parts: the indirect effect of X on Y, reflected by ab , and the direct effect of X on Y with the effect of the mediator removed, reflected by c' .

Of the remaining two types of methods to test mediation, the first category is based on the difference in coefficient method. The approach compares coefficients between the independent and dependent variable before and after including the effects of a mediating variable ($c - c'$). The final approach, the product of coefficients method, tests the significance of the mediating variable by dividing the estimate of the indirect effect (ab) by its standard error, and comparing this value to a standard normal distribution to test for significance.

In this paper we adopt the product of coefficients approach to test the mediating effects of cognitive variables on the genetic association of the number of risk alleles on behavioural ADHD ratings. There are multiple formulas to estimate the standard error (SE) of the mediated (ab) effect (see MacKinnon et al., 2002). We adopt the most frequently used formula, derived by Sobel: $\sqrt{(a^2 * SEb^2) + (b^2 * SEa^2)}$ (where SEa refers to the standard error of a and SEb refers to the standard error of b) (MacKinnon et al., 2002). As stated above, ab is then divided by its standard error, and tested for significance using MacKinnon's z distribution. For these analyses, we adopted an alpha value of 0.05, for which a z value that does not lie between 1.96 to -1.96 is considered significant.

4.4 Results

4.4.1 Association analyses

Association findings are listed in Table 4.1. Our strongest association was found when looking at total tests of association (AT) between RTV and a SNP in 5HT2A (rs7984966) ($p = 0.007$). There was also a nominal association with another 5HT2A SNP (rs7322347) and inattention ($p = 0.01$). For both genetic markers, the risk allele was implicated as the T-allele. Heightened RTV was also nominally associated with one CDH13 SNP (rs11646411, $p = 0.03$; G-allele implicated as the risk allele). One of the SNPs in SLC6A2 (rs3785143) showed a nominal AT association with CE ($p = 0.03$) (T-allele implicated as the risk allele).

Taking into account the presence of population stratification effects, and therefore using the AW results, nominal AW associations were found for the other SLC6A2 SNP (rs3785157) and both hyperactivity-impulsivity ($p = 0.04$) and CE ($p = 0.05$) (T-allele implicated as risk allele). In

addition we found nominal associations between the HTR1B gene and increased CE ($p = 0.02$) (the G-allele in rs6296 implicated as the risk allele).

Although, we found an AT association with the TPH2 SNP (rs1843809), as there was also evidence for population stratification effects, the AT association should be ignored in favour of the within-test (AW) estimate (which was non-significant).

None of these associations withstood correction for the number of SNPs examined (0.003). However, both SNPs in SLC6A2 remain of potential interest because of overlapping associations: in addition to associations already noted ($p \leq 0.05$) SLC6A2 SNPs showed further (though nominal ($p = 0.09$)) associations with hyperactivity-impulsivity (rs3785143) and inattention (rs3785157). In addition, 5HT2A is of potential interest because of overlapping associations within the rs7984966 SNP, with a further nominal ($p = 0.09$) association with inattention; as well as multiple associations across the two SNPs investigated. Therefore we decided to test mediation models for these overlapping associations with both SLC6A2 SNPs and the 5HT2A SNP, rs7984966.

Table 4.1 QTTDT association analysis in a population-based twin sample

ADHD MARKERS		HYPERACTIVITY-IMPULSIVITY			INATTENTION			REACTION TIME VARIABILITY			COMMISSION ERRORS		
Gene (marker)	df	AP	AT	AW	AP	AT	AW	AP	AT	AW	AP	AT	AW
CDH13 (rs6565113)	1	0.47	0.82	0.46	0.73	0.54	0.55	0.92	0.62	0.89	0.43	0.55	0.33
CDH13 (rs11646411)	1	0.82	0.39	0.87	0.58	0.85	0.66	0.17	0.03	0.73	0.65	0.77	0.60
CNTFR (rs7036351)	1	0.22	0.45	0.50	0.18	0.51	0.36	0.54	0.31	0.95	0.46	0.62	0.38
COMT VNTR	1	0.60	0.57	0.44	0.71	0.16	0.47	0.17	0.57	0.58	0.52	0.26	0.74
DAT1_3 VNTR	2	0.15	0.76	0.64	0.69	0.29	0.93	0.06	0.75	0.41	0.56	0.34	0.39
DAT1_8 VNTR	2	0.04	0.76	0.09	0.45	0.66	0.83	0.59	0.40	0.25	0.32	0.79	0.49
DRD4 VNTR	3	0.84	0.76	0.99	0.17	0.83	0.22	0.80	0.28	0.41	0.17	0.93	0.41
HTR1B (rs6296)	1	0.95	0.81	0.87	0.64	0.80	0.59	0.81	0.48	0.92	0.05	0.23	0.02
MAOA (rs6323)	NT	NT	NT	0.24	NT	NT	0.22	NT	NT	0.11	NT	NT	0.31
SLC6A2 (rs3785143)	1	0.09	0.66	0.09	0.36	0.80	0.48	0.23	0.12	0.93	0.41	0.03	0.10
SLC6A2 (rs3785157)	1	0.03	0.71	0.04	0.07	0.88	0.09	0.96	0.53	0.74	0.03	0.95	0.05
SNAP25 (rs1051312)	1	0.03	0.23	0.24	0.15	0.50	0.32	0.93	0.18	0.48	0.93	0.08	0.39
SNAP25 (rs6077690)	1	0.29	0.58	0.54	0.40	0.69	0.35	0.79	0.93	0.85	0.31	0.88	0.33
TPH2 (rs1843809)	1	0.14	0.96	0.22	0.03	0.05	0.25	0.77	0.31	0.83	0.54	0.90	0.55
5HT2A (rs7322347)	1	0.10	0.38	0.29	0.45	0.01	0.69	0.41	0.14	0.17	0.93	0.58	0.88
5HT2A (rs7984966)	1	0.11	0.75	0.12	0.11	0.09	0.40	0.65	0.007	0.43	0.72	0.81	0.82

Note: df: difference in degrees of freedom between the null and alternative models; AP: Test for population stratification; AT: Total Test of Association; AW: Within-Test of Association; p values ≤ 0.05 are in bold; p values ≤ 0.10 are in italic typeface; NT = Not tested;

4.4.2 Testing candidate mediating pathways

QTD associations were observed for the SLC6A2 SNP (rs3785157) with CE and both ADHD symptom domains. Therefore we tested two candidate pathways using two models with inattention/hyperactivity-impulsivity modelled as the outcome in alternative models. As the QTD associations with rs3785157 were found using the AW test, within-pair differences for phenotype and genotype (specifically risk alleles) were used in the regression tests for mediation. Although we found overlapping associations with the other SLC6A2 SNP (rs3785143) and both hyperactivity-impulsivity and CE, these could not be tested in a mediation model as associations in QTD were mixed (associations with CE obtained using AT test, and association with hyperactivity-impulsivity obtained using AW test).

Inattention and RTV showed associations with the 5HT2A SNP rs7984996, and as both associations were found in the AT test, differences scores were not used for the regression analysis. Instead, number of risk alleles and phenotype data was used, and the 'cluster' command employed to account for the genetic relationship within twin pairs.

Testing CE as mediating the association between SLC6A2 and hyperactivity-impulsivity: In the first step of the regression analysis, we tested pathway *a*, and found that the SLC6A2 SNP rs3785157 significantly predicted CE ($\beta = 7.53$, standard error (SE) = 3.18, $p = 0.02$). In the second regression step of the regression analysis, we tested pathway *b*, and found that CE significantly predicted hyperactivity-impulsivity ($\beta = 0.001$, SE = 0.001, $p = 0.05$). The indirect effect (*ab*) is estimated at 0.00753, and the SE of *ab* is estimated at 0.008 ($\sqrt{(b^2 * SEa^2) + (a^2 * SEb^2)}$). The *z* statistic was estimated at 0.92 with a *p* value of 0.36 (*ab/SEab*). Therefore, the mediated effect of CE on the association between SLC6A2 and hyperactivity-impulsivity was not significant.

Testing CE as mediating the association between SLC6A2 and inattention: The first step in this mediation model is a repeat of the first step in the previous model (as the SNP and mediator are the same). In the second step of the regression analysis, CE significantly predicted inattention ($\beta = 0.002$, SE = 0.001, $p = 0.001$). The mediated effect of CE on the association between SLC6A2 and inattention was not significant (*ab* = 0.015, *z* = 1.53, $p = 0.13$).

Testing RTV as mediating the association between 5HT2A and inattention: The two regression analyses revealed that the 5HT2A SNP rs7984966 significantly predicted RTV (pathway *a*: $\beta = 0.09$, $SE = 0.03$, $p = 0.001$), and that RTV significantly predicted inattention (pathway *b*: $\beta = 0.18$, $SE = 0.02$, $p < 0.001$). The mediated effect of RTV on the association between 5HT2A and inattention was significant ($ab = 0.016$, $z = 2.85$, $p = 0.004$). The proportion of the effect of rs7984966 on inattention accounted for by RTV was 49% ($((c-c')/c)*100$) ($c = 0.0332$, $c' = 0.017$).

PART B: AN ADHD-PROBAND AND CONTROL SIBLING-PAIR SAMPLE

4.5 Methodology

4.5.1 Sample and procedure

Participants were recruited from the International Multicentre ADHD Genetics (IMAGE) project. The IMAGE project is an international collaborative study that aims to identify genes which increase the risk for ADHD, using a combination of categorical and quantitative trait approaches. Participants were recruited from specialist clinics in Belgium, Germany, Holland, Ireland, Israel, Spain, Switzerland and the United Kingdom. All participants were of European Caucasian descent and aged six to 18 years. All probands had a clinical diagnosis of ADHD-C subtype and had at least one full sibling (unselected for clinical phenotype) and biological parents available for ascertainment of clinical information and DNA. Exclusion criteria applying to both probands and siblings included an IQ of less than 70, autism, epilepsy, general learning difficulties, brain disorders, and any genetic or medical disorder associated with externalizing behaviours that might mimic ADHD.

Families were invited to the Research Centre for cognitive assessments, and a parent interview. A minimum of a 48-hour medication-free period prior to testing was required for cognitive testing. The ADHD proband and sibling(s) for each family were tested simultaneously by trained researchers in separate testing rooms. Short breaks were given as required, and the total length of the test session was 2.5 to 3 hours.

4.5.2 Measures

IQ was assessed using four subtests from the Wechsler Intelligence Scales for Children (Third Edition) (Wechsler, 1991). Cognitive data was obtained using the go/no-go (GNG) task (Borger & van der Meere, 2000; Kuntsi, Andreou et al., 2005; van der Meere et al., 1995) and the fast task (Andreou et al., 2007; Kuntsi, Andreou et al., 2005; Kuntsi, Rogers et al., 2006); (see section 3.3.2.3 and 3.3.2.4 for more details).

To enable a direct comparison to the variables used in the analysis on the population-based sample, we aimed to create similar summed scores across two tasks or conditions as follows: unstandardised RTV across the slow condition of the GNG task and baseline condition of the fast task; and percentage of CE across slow and fast conditions of the GNG task. However, due to missing data and some sites not administering both tasks, instead of creating a summed score (which requires data across both conditions/tasks) we created a mean score for individuals that had two scores, and for individuals with only one score used this for analysis. As mean scores were created for some participants, scores were standardised before deriving the final scores. We had genotyping data and regressed (for age, sex and IQ) RTV and CE scores for 454 and 413 individuals.

Parents and teachers were asked to complete the Long Versions of Conners' Parent and Teacher Rating Scales (Conners et al., 1998a, 1998b). From both scales, we used the nine-item inattention and nine-item hyperactivity-impulsivity DSM-IV ADHD symptom subscales, obtaining summed parent and teacher ratings on the corresponding subscales. Summed inattention and hyperactivity-impulsivity subscale ratings (regressed for age and sex), were available for 767 and 772 individuals, respectively.

The dataset was then supplemented with data from participants across other sites that did not participate in cognitive testing, but had genotyping data and behavioural ADHD ratings from the Conners Rating Scales'. The final dataset used in this study consisted of 674 DSM-IV combined ADHD subtype probands with 871 siblings, with summed inattention and hyperactivity-impulsivity subscale ratings regressed for age and sex, available for 1466 and 1471 individuals, respectively.

4.5.3 Genotyping and analyses

Information on DNA collection, genotype assays, and quality control are described elsewhere (see Brookes et al., 2006). From our previous analysis in the population-based twin sample, we decided to undertake a detailed analysis of SNPs spanning SLC6A2 and 5HT2A. Five markers, of a total of 75, did not conform to Hardy-Weinberg equilibrium ($p < 0.01$) and were dropped from further analysis. Therefore a total of 37 SNPs from SLC6A2 and 33 SNPs from 5HT2A were taken forward for analysis.

Genetic association analysis was conducted using QTDT (see section 4.3.5.1 for more details), and on similarly derived variables as used in previous quantitative and molecular genetic analysis on the population-based twin sample (see section 4.5.2).

4.6 Results

A total of 20 valid nominal AT or AW associations ($p \leq 0.05$) were found for SLC6A2 (see Table 4.2). Associations were found across all variables, but the majority of associations were with CE. The strongest associations were found for hyperactivity-impulsivity (rs36017 and rs2279805; $p = 0.004$ (AW)). The only overlapping SLC6A2 SNP from this and the previous analysis was rs3785143, which was significantly associated in the clinical sample with inattentive ratings ($p = 0.04$), but associated with CE and hyperactivity-impulsivity ratings in our population-based sample. The only SNP that showed valid associations across both cognitive and behavioural data was rs156652, which showed significant associations with both ADHD symptom domains and CE. As the associations were obtained across a mixture of association tests (AT and AW), this could not be taken forward and tested for mediating). None of these associations withstood Bonferroni correction for the number of SNPs in SLC6A2 examined.

Five AW associations with 5HT2A SNPs were found across hyperactivity-impulsivity and inattention, but should be ignored as there were no significant AP effects (see Table 4.3). There were no significant AT or AW associations with RTV or CE. The only overlapping 5HT2A SNP across both analysed samples, rs7322347, did not contribute to any significant associations in the clinical sample, although this was associated with inattention in our population-based sample.

Table 4.2 QTDt association analysis of SNPs spanning SLC6A2 in clinical proband and sibling sample

Marker	df	HYPERACTIVITY- IMPULSIVITY			INATTENTION			REACTION TIME VARIABILITY			COMMISSION ERRORS		
		AP	AT	AW	AP	AT	AW	AP	AT	AW	AP	AT	AW
rs7201099	1	0.48	0.24	0.19	0.63	0.81	0.85	0.35	0.05	0.44	0.55	0.59	0.99
rs4783899	1	0.26	0.50	0.73	0.24	0.02	0.44	0.76	0.46	0.45	0.27	0.10	0.60
rs168924	1	0.64	0.23	0.68	0.27	0.28	0.90	0.98	0.24	0.41	0.68	0.87	0.69
rs1805064	1	NT	0.88	NT	NT	0.37	NT	NT	1.00	NT	NT	1.00	NT
rs3785143	1	0.05	0.53	0.06	0.009	0.92	0.04	0.16	0.91	0.29	0.77	0.81	0.71
rs192303	1	0.78	0.13	0.42	0.86	0.41	0.68	0.39	0.04	0.34	0.94	0.46	0.54
rs41154	1	0.36	0.93	0.47	0.43	0.46	0.97	0.82	0.22	0.29	0.54	0.28	0.68
rs1805065	1	0.54	0.53	0.38	0.66	0.78	0.60	0.36	0.05	0.40	0.26	0.95	0.50
rs13306041	1	NT	0.08	NT	NT	0.24	NT	NT	0.31	NT	NT	0.60	NT
rs187715	1	0.77	0.88	0.75	0.40	0.46	0.26	0.67	0.28	0.71	0.01	0.17	0.005
rs15568343	1	0.12	0.75	0.17	0.24	0.75	0.52	NT	0.27	NT	NT	0.77	NT
rs36024	1	0.49	0.73	0.78	0.93	0.80	0.92	0.30	0.94	0.44	0.59	0.56	0.95
rs187714	1	0.31	0.84	0.38	0.43	0.50	0.90	0.88	0.17	0.26	0.54	0.24	0.64

rs36023	1	0.58	0.92	0.64	0.36	0.20	0.12	0.75	0.74	0.65	0.70	0.10	0.33
rs36021	1	0.32	0.16	0.09	0.07	0.65	0.11	0.48	0.02	0.03	0.98	0.82	0.85
rs3785152	1	0.09	0.74	0.14	0.40	0.52	0.29	0.62	0.83	0.62	0.11	0.97	0.26
rs1814269	1	0.24	0.56	0.22	0.42	0.71	0.40	0.73	0.20	0.48	0.98	0.02	0.08
rs36017	1	0.03	0.07	0.004	0.07	0.22	0.03	0.42	0.47	0.98	0.99	0.45	0.55
rs1805066	1	0.15	0.46	0.62	0.27	0.64	0.63	NT	0.20	NT	NT	0.59	NT
rs11568340	1	NT	0.73	NT	NT	0.38	NT	NT	1.00	NT	NT	1.00	NT
rs2840109	1	NT	0.11	NT	NT	0.40	NT	NT	1.00	NT	NT	1.00	NT
rs10521329	1	0.18	0.37	0.11	0.50	0.12	0.13	0.17	0.80	0.41	0.46	0.04	0.05
rs3785155	1	0.32	0.41	0.19	0.38	0.04	0.04	0.21	0.33	0.11	0.92	0.07	0.21
rs36013	1	0.84	0.79	0.96	0.83	0.77	0.72	NT	0.14	NT	NT	0.87	NT
rs5564	1	0.76	0.40	0.43	0.47	0.85	0.51	0.30	0.85	0.58	0.14	0.03	0.54
rs11568324	1	0.02	0.63	0.02	0.35	0.31	0.18	NT	0.64	NT	NT	0.46	NT
rs1805068	1	0.49	0.65	0.41	0.54	0.63	0.90	NT	0.67	NT	NT	0.98	NT
rs1861647	1	0.16	0.22	0.06	0.21	0.92	0.32	0.83	0.26	0.50	0.84	0.84	0.77
rs2279805	1	0.03	0.06	0.004	0.06	0.20	0.02	0.21	0.23	0.93	0.95	0.46	0.54
rs1566652	1	0.01	0.28	0.008	0.03	0.57	0.05	0.88	0.48	0.67	0.71	0.05	0.20

rs36010	1	0.78	0.75	0.99	0.83	0.68	0.66	NT	0.12	NT	NT	0.84	NT
rs8047672	1	0.12	0.13	0.03	0.23	0.04	0.02	0.24	0.35	0.85	0.39	0.06	0.05
rs5569	1	0.06	0.23	0.03	0.19	1.00	0.32	0.88	0.44	0.66	0.87	0.49	0.54
rs36009	1	0.71	0.05	0.28	0.41	0.06	0.47	0.99	0.79	0.86	0.38	0.30	0.90
rs1800887	1	0.27	0.84	0.34	0.36	0.23	0.90	0.27	0.67	0.29	0.18	0.04	0.02
rs36008	1	0.64	0.46	0.41	0.40	0.47	0.27	NT	0.21	NT	NT	0.51	NT
rs5560	1	0.10	0.67	0.36	0.05	0.56	0.06	NT	0.99	NT	NT	0.97	NT
rs2242447	1	0.32	0.74	0.61	0.58	0.70	0.88	0.62	0.40	0.34	0.98	0.005	0.04

Note: df = difference in degrees of freedom between the null and alternative models; AP: Test for population stratification; AT: Total Test of Association; AW: Within-Test of Association; p values ≤ 0.05 are in bold typeface; NT = Not tested;

Table 4.3 QTDT association analysis of SNPs spanning 5HT2A in clinical proband and sibling sample

Marker	df	HYPERACTIVITY- IMPULSIVITY			INATTENTION			REACTION TIME VARIABILITY			COMMISSION ERRORS		
		AP	AT	AW	AP	AT	AW	AP	AT	AW	AP	AT	AW
rs3803189	1	0.64	0.20	0.23	0.15	0.14	0.04	0.10	0.40	0.57	0.20	0.89	0.41
rs3125	1	0.68	0.17	0.21	0.15	0.12	0.03	0.10	0.40	0.57	0.20	0.89	0.41
rs6314	1	0.24	0.82	0.31	0.40	0.21	0.14	0.13	0.79	0.39	0.81	0.43	0.68
rs7322347	1	0.66	0.48	0.42	0.40	0.60	0.33	0.33	0.65	0.73	0.08	0.55	0.47
rs1923882	1	0.35	0.27	0.15	0.13	0.06	0.02	0.54	0.61	0.93	0.30	0.74	0.33
rs977003	1	0.74	0.44	0.44	0.42	0.51	0.30	0.37	0.85	0.63	0.06	0.61	0.37
rs9567735	1	0.48	0.37	0.26	0.87	0.86	0.99	0.25	0.59	0.61	0.31	0.83	0.56
rs6561333	1	0.28	0.23	0.11	0.43	0.64	0.37	0.93	0.65	0.80	0.02	0.44	0.28
rs1923884	1	0.47	0.44	0.29	0.83	0.94	0.90	0.25	0.59	0.61	0.31	0.83	0.56
rs1923886	1	0.28	0.19	0.09	0.54	0.59	0.42	0.53	0.50	0.36	0.04	0.34	0.44
rs1745837	1	0.15	0.17	0.05	0.41	0.55	0.32	0.61	0.26	0.67	0.26	0.40	0.88
rs9316232	1	0.34	0.26	0.14	0.98	0.50	0.64	0.10	0.63	0.41	0.08	0.45	0.49
rs9316233	1	0.66	0.42	0.38	0.75	0.36	0.40	0.03	0.06	0.83	0.63	0.37	0.32
rs659734	1	0.84	0.30	0.38	0.95	0.23	0.43	0.21	0.47	0.70	0.96	0.83	0.85
rs1928042	1	0.34	0.99	0.48	0.42	0.67	0.74	0.22	0.46	0.16	0.13	0.32	0.70

rs2770296	1	0.18	0.94	0.30	0.85	0.83	0.99	0.56	0.93	0.62	0.19	0.75	0.46
rs9316235	1	0.42	0.57	0.85	0.86	0.51	0.56	0.88	0.68	0.86	0.49	0.27	0.76
rs582385	1	0.33	0.37	0.18	0.87	0.82	0.79	0.53	0.57	0.40	0.12	0.92	0.30
rs1928040	1	0.60	0.92	0.76	0.85	0.79	0.96	0.87	0.23	0.34	0.61	0.80	0.59
rs731779	1	0.57	0.43	0.34	0.50	0.86	0.73	0.22	0.27	0.92	0.39	0.65	0.79
rs985934	1	0.22	0.43	0.15	0.96	0.59	0.74	0.44	0.29	0.88	0.53	0.96	0.68
rs927544	1	0.22	0.72	0.52	0.84	0.45	0.72	0.53	0.26	0.74	0.56	0.42	0.86
rs9534505	1	0.61	0.68	0.91	0.61	0.88	0.77	0.06	0.30	0.43	0.86	0.95	0.92
rs4941573	1	0.64	0.84	0.64	0.75	0.71	0.98	0.88	0.07	0.24	0.30	0.63	0.29
rs1328684	1	0.74	0.53	0.50	0.95	0.74	0.86	0.25	0.68	0.27	0.32	0.82	0.39
rs6305	1	0.57	0.15	0.16	0.24	0.09	0.04	0.70	0.58	0.88	0.76	0.89	0.76
rs2296973	1	0.39	0.10	0.08	0.71	0.35	0.70	0.05	0.07	0.93	0.88	0.67	0.84
rs2070037	1	0.49	0.32	0.24	0.57	0.77	0.83	0.05	0.07	0.99	0.64	0.49	0.84
rs6313	1	0.64	0.94	0.69	0.83	0.54	0.79	0.72	0.07	0.32	0.43	0.62	0.37
rs6311	1	0.63	0.97	0.71	0.82	0.62	0.86	0.73	0.06	0.28	0.43	0.54	0.32
rs1328685	1	0.57	0.18	0.18	0.86	0.08	0.30	0.66	0.75	0.92	0.70	0.17	0.21
rs4142900	1	0.92	0.50	0.72	0.92	0.82	0.82	0.66	0.07	0.11	0.24	0.51	0.20

Note: df = difference in degrees of freedom between the null and alternative models; AP: Test for population stratification; AT: Total Test of Association; AW: Within-Test of Association; p values ≤ 0.05 are in bold typeface; NT = Not tested;

4.7 Discussion

Using a large population-based twin sample, this study examined the molecular genetic correlates of a number of quantitative ADHD-related traits suggested as potential ADHD endophenotypes and viable targets for molecular genetic investigation by previous quantitative genetic analyses conducted on the same sample, with selected markers previously nominated as ADHD risk genetic variants. Overlapping findings were then subjected to test for mediation versus pleiotropic effects, and a detailed SNP analysis in an independent clinical sample.

The analysis on a population-based twin sample yielded several nominal associations, although none withstood correcting for multiple testing. In the population twin sample we observed a nominal association between CE and the G allele of the HTR1B SNP rs6296, in line with previous meta-analyses that reported an association with the same allele and clinical ADHD (Faraone et al., 2005; Gizer et al., 2009). In addition, we observed a nominal association between RTV and CDH13, an interesting candidate gene for ADHD as it is the only common finding across existing GWAS (Franke, Neale, & Faraone, 2009), in both child (Lasky-Su, Neale et al., 2008) and adult (Lesch et al., 2008; Neale et al., 2008) clinical samples. Moreover, this gene lies within a region (chromosome 16) that was highlighted in a recent meta-analysis of genome-wide linkage scans of ADHD (Zhou, Dempfle et al., 2008). Tested markers were selected based on previous candidate gene association studies of ADHD, and so these associations observed with ADHD-related phenotypes extend previous findings with clinical ADHD; however, none of these associations withstood correction for multiple testing.

Nominal associations were also found for two SNPs in SLC6A2, which is a compelling site for candidate gene studies and holds significant promise for increasing ADHD susceptibility as pharmacological studies have reported decreased ADHD behaviours with atomoxetine, a norepinephrine transporter antagonist (Xu, Knight et al., 2005). Two studies investigating SNPs spanning the length of the SLC6A2 gene reported associations with ADHD, although significant associations with specific SNPs were not consistent across both studies (Guan et al., 2009; Xu, Knight et al., 2005). Although the most recent and comprehensive meta-analysis did not yield a significant association between SLC6A2 and ADHD (Gizer et al., 2009), the authors stated that the majority of studies investigating SLC6A2 evaluated different SNPs and that there was only a

sufficient number of studies for two SNPs to be investigated with meta-analytical techniques. The authors therefore concluded that SLC6A2 may still be of significance and warranted further research (Gizer et al., 2009).

In the analysis on the population-based sample we identified the T allele of the SLC6A2 SNP, rs3785157, as the risk allele, in line with some studies (Bobb et al., 2005), but opposing others (Xu, Knight et al., 2005). We also found associations with rs3785143 in SLC6A2, which was reported as a novel finding with ADHD-C subtype (Brookes et al., 2006). In addition, the pattern of associations with SLC6A2 was in line with our previous quantitative genetic findings: one SNP was associated with both ADHD behavioural dimensions and CE; and both SNPs that were associated with CE, were not associated with RTV. The association of both SLC6A2 SNPs with CE are in line with a recent study of Korean children with ADHD, which found an association with SLC6A2 and CE (Song, Jhung, Song, & Cheon, 2011).

Our strongest association in our analysis on a population-based sample was between a SNP in 5HT2A, rs7984966, and RTV. This SNP and a further SNP in 5HT2A (rs7322347) were also associated with inattention. The pattern of findings - overlapping associations with RTV and inattention, and non-overlapping associations with RTV and CE - is in line with the results from our quantitative genetic analysis (chapter 3). These SNPs have been associated both in single marker and haplotype (multiple markers) analyses with ADHD-C subtype in both children and adults (Ribases et al., 2009). However, our associations were with the opposite alleles to those reported in the previous literature (Ribases et al., 2009). As this is the first attempt to replicate associations with the identical specific SNPs, further research in independent samples are needed to clarify the direction of the observed associations. However, it is interesting to note that this finding in the opposite direction to the expected effect, may mirror DRD4 findings in the literature, which suggest that the *absence* of the ADHD risk allele is associated with superior RTV performance findings (Bellgrove et al., 2008; Kebir & Joober, 2011; Kebir et al., 2009) (see section 1.6.4.2).

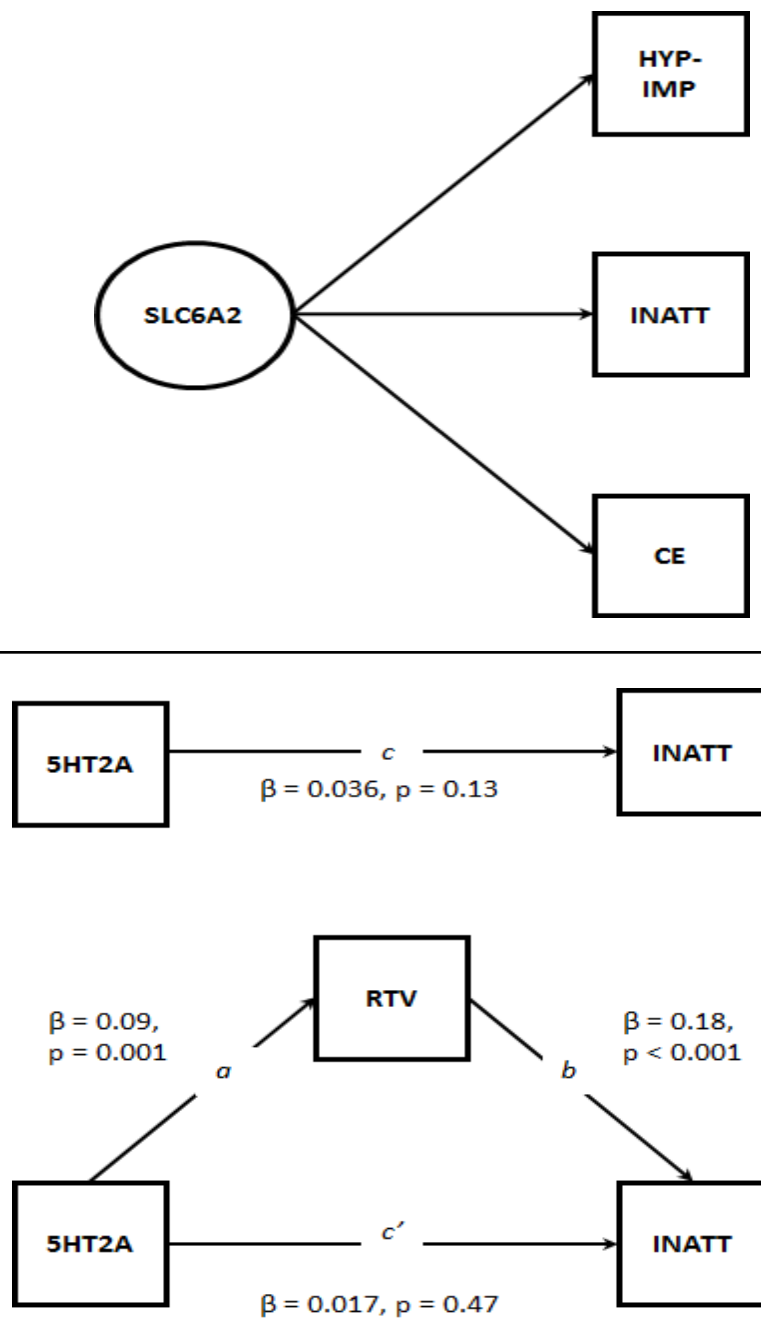
A related important new direction of research is testing whether endophenotypes are involved in pathways from genes to behaviour (Kendler & Neale, 2010; Langley et al., 2010), which may also uncover the functional roles of genes involved. The quantitative findings that emerged in

chapter 3 implicated the CE-ADHD association as reflecting genetic influences that CE shares both with inattention and hyperactive-impulsive symptoms. Overlapping associations from molecular genetic analysis of the same sample with the same variables are in line with these findings. The overlapping nominal associations suggest that rs3785157 in SLC6A2 may be a potential genetic candidate contributing to the association of CE with both ADHD behavioural dimensions. Further analyses did not find any evidence of mediation, and so these nominal associations reflect pleiotropy (see Figure 4.2).

The overlapping nominal associations for a SNP in 5HT2A (rs7322347) with RTV and inattention implicate it as a potential candidate marker contributing to our quantitative findings that the RTV-ADHD association reflects largely genetic influences shared between RTV and inattention (chapter 3). Mediation analysis indicated that RTV mediated a substantial proportion of the effect of 5HT2A on inattention symptoms. Partial mediation is entirely in keeping with expectations for a common complex behavioural trait, where multiple risk factors are involved, and is consistent with theoretical models that hypothesise that cognitive factors lie of the pathway between genes and ADHD.

A detailed SNP analysis of SNPs spanning SLC6A2 in an independent clinical sample yielded a number of nominal associations, particularly for CE, although none withstood correction for multiple testing. The only overlapping SLC6A2 SNP across both samples, rs3785143, was associated with hyperactivity-impulsivity and CE in the population-based sample and inattention in our clinical sample. Although the replication is not with the same variables, this may potentially reflect a cross-domain SNP, or chance findings. In addition, analysis in the clinical sample with one SLC6A2 SNP (rs1566652) displayed a similar trio of associations (overlapping associations with hyperactivity-impulsivity, inattention and CE) as observed for rs3785157 in the population samples. None of these associations withstood correction for the number of markers tested.

Figure 4.2 Model depicting the pleiotropic effects of SLC6A2 (upper model) and the direct effects of 5HT2A on inattention and the indirect effect via RTV (lower model) in population-based twin sample



Note: Abbreviations: HYP-IMP: Hyperactivity-impulsivity; INATT: inattention; RTV: Reaction time variability; CE: Commission errors;

An analysis of SNPs spanning 5HT2A in the clinical sample yielded no significant associations. Accordingly we were unable to replicate the finding that emerged in our population-based sample with rs7322347 (the only overlapping SNP in 5HT2A tested in both samples). Furthermore, in our population-based sample we found suggestive evidence of a causal pathway from this overlapping SNP, via RTV, to inattention. As replication failed at the initial stage of association, we were unable to test for mediation, and so further studies are required to verify this finding. Moreover, future studies should extend investigations to other genetic markers and cognitive processes that might reflect multiple gene-cognitive pathways. This is an important step if we wish to identify causal processes that might become the target of prevention and treatment interventions for ADHD.

Common with most studies incorporating quantitative assessments within molecular genetic investigations, power to detect genetic association was limited, particularly so for cognitive measures in our clinical sample. The genetic associations need to be treated with considerable caution, since they fall well short of genome-wide levels of significance (Dudbridge & Gusnanto, 2008). Furthermore, some of the allelic specific associations we identified were not in the same direction as predicted by the previous literature. Despite these limitations, we did find nominal associations with previously implicated ADHD susceptibility genes and psychometrically robust ADHD-related phenotypes, selected on the basis of quantitative analysis. The consistency in the pattern of findings from quantitative (chapter 3) and molecular genetic analyses was initially encouraging, suggesting that some previously observed genetic associations identified in clinical samples can be extrapolated to quantitative measures of ADHD behaviours in the general population. However, none of the associations withstood correction for multiple testing, nor were replicated in the subsequent analysis on an independent clinical sample. Reasons for failure to replicate may include differences related to sample ascertainment: clinical samples are more prone to referral bias, ADHD severity and comorbidity. In addition, the age range of the clinical sample was much broader than the population-based sample, and had a much higher preponderance of male participants (two-thirds were male, compared to an equal gender breakdown in the population-based sample). Despite these potentially sources for non-replication, the failure to replicate across independent samples, emphasise the need for future studies.

CHAPTER 5 GENETIC OVERLAP BETWEEN ADHD BEHAVIOURS AND AUTISTIC-LIKE TRAITS

5.1 Abstract

Attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorders (ASDs) frequently co-occur. An increasing number of studies have compared clinical groups to identify common and disorder-specific neuroanatomical and neuropsychological correlates, to elucidate shared and unique pathways underlying ADHD and ASDs. However, as a result of current diagnostic exclusionary criteria that prohibit a dual diagnosis, little is known about the underlying causes of the covariation of these disorders. Twin studies assessing shared and specific aetiological factors of quantitative assessments of ADHD behaviours and autistic-like traits (ALTs) suggest substantial genetic overlap. However, existing twin studies have failed to take into account the modest distinct aetiologies of ADHD symptoms, and the great genetic heterogeneity of the symptom subscales of ALTs. This study aimed to clarify the phenotypic and genetic relationship between ADHD behaviours and ALTs, distinguishing between symptom subscales. Moreover, we aimed to investigate whether ADHD-related cognitive impairment factors showed a differential relationship with ALT subscales, and whether genetic effects underlying cognitive performance measures are shared or distinct from those shared between ADHD and ASD symptom subscales. Multivariate structural equation modelling was conducted on behavioural ratings and cognitive-experimental measures obtained from a population-based twin sample of 1312 children aged between seven and 10. Non-social ALTs showed low phenotypic correlation with hyperactivity-impulsivity (0.11), and partially overlapping genetic effects ($r_G = 0.20$). In contrast, social ALTs correlated moderately and equally with both inattention and hyperactivity-impulsivity (around 0.30). Moreover, social ALTs showed equally substantial genetic overlap with both inattention ($r_G = 0.52$) and hyperactivity-impulsivity ($r_G = 0.44$). Reaction time variability (RTV) was phenotypically associated with social ALTs (0.18), and both phenotypes showed moderate shared genetic effects ($r_G = 0.32$). Further analyses suggested that the shared genetic effects between inattention and social ALTs were largely independent of genetic effects shared with RTV, despite this being a common underlying cognitive impairment. In conclusion, our findings suggest that social ALTs underlie the previously observed association between ALTs and ADHD behaviours, equally with both inattention and hyperactivity-impulsivity. These novel findings underline that the separation of

both ADHD behaviours on the one hand, and ALTs on the other, may help clarify the link between ASD and ADHD, and elucidate shared versus unique neuropsychological pathways underlying these neurodevelopmental behaviours.

5.2 Introduction

Despite the observed high co-occurrence of attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorders (ASDs) (Rommelse et al., 2010; Simonoff et al., 2008) (see section 1.7.2), current diagnostic criteria prohibits a dual diagnosis, although this is likely to be amended in upcoming diagnostic revisions (Coghill & Seth, 2011). However, as a result of the diagnostic exclusionary criteria, little is known about the underlying causes of the covariation of these disorders (Ronald et al., 2008).

Both ADHD behaviours and autistic symptoms can be viewed as continuously distributed traits (Chen et al., 2008; Corbett, Constantine, Hendren, Rocke, & Ozonoff, 2009; Dawson et al., 2002; Lubke et al., 2009). Consequently, twin studies have explored shared and specific aetiological influences of quantitative assessments of ADHD symptoms and autistic-like traits (ALTs). Analysis of ratings on a UK population-based twin sample (Twins' Early Development Study (TEDS)) at age 8, yielded significant phenotypic correlations (around 0.50) between ADHD symptoms and ALTs (Ronald et al., 2008). Substantial common genetic influences were found whether assessing co-variation throughout the population, at the quantitative extreme, or adopting a categorical approach (genetic correlations (r_G) > 0.50) (Ronald et al., 2008). These findings were consistent across genders and informants. A similarly high genetic correlation was obtained for self-report symptom (inattentive and impulsivity symptoms only (hyperactivity symptoms not included)) ratings in adulthood (0.72) (Reiersen, Constantino, Grimmer, Martin, & Todd, 2008). In one of the largest twin study to date (10,895 twin pairs aged nine and 12 screened for ASDs and associated conditions using parental telephone interviews), a strong overlapping genetic liability was observed between these two disorders ($r_G = 0.87$) (Lichtenstein et al., 2010). Taken together, these findings suggest that ALTs and ADHD behaviours are modestly correlated and share a common genetic aetiology.

A limitation of the above studies is that they do not take into account the great genetic heterogeneity observed within the symptom subscales of ASD (Dworzynski et al., 2009; Ronald, Happe, Bolton et al., 2006; Ronald et al., 2005; Ronald, Happe, Price et al., 2006) and the partially distinct aetiologies of ADHD behaviours (Greven, Rijdsdijk et al., 2011; McLoughlin et al., 2007). This issue was partly addressed in a sample of two-year-old twins that decomposed ALTs into social and non-social subscale components (Ronald, Edelson et al., 2010). Despite the young age of the sample, the phenotypic and genetic covariation of ADHD behaviours and ALTs was evident, although slightly lower than observed in the aforementioned studies, suggestive of a developmental increase. Moreover, both social and non-social ALT subscales contributed equally to the phenotypic covariation and aetiological influences shared with ADHD behaviours (Ronald, Edelson et al., 2010). However, no study has investigated associations with ADHD behaviours separated into inattention and hyperactivity-impulsivity.

An extensive review on the link between ASD and ADHD highlighted that these two disorders have often been studied in isolation from one another (Rommelse et al., 2011). The authors suggested that molecular genetic studies searching for pleiotropic genes should target common endophenotypes (see section 1.6 and Rommelse et al., (2011) for more details). A plethora of studies have investigated cognitive deficits in ADHD and ASD separately, and the past decade has witnessed an increase in studies comparing cognitive profiles across these clinical groups. In contrast, comparatively few studies have also compared single- with dual-diagnosis (ADHD+ASD) groups (Rommelse et al., 2011). Exploring neuropsychological deficits common to both ADHD and ASD may increase understanding of shared gene-cognitive pathways (Rommelse et al., 2011). Moreover, the identification of disorder-specific cognitive impairments may facilitate differentiation of these disorders, and be employed as disorder-specific targets in candidate gene association studies (Johnson, Robertson et al., 2007).

As previously reviewed (see section 1.6), reaction time variability (RTV) reflecting attentional lapses, has emerged as one of the strongest cognitive ADHD endophenotypes (Kuntsi & Klein, 2011). Studies that have investigated the non-specificity of RTV with ADHD versus ASD, report mixed findings. One study suggested that high RTV may be specific to ADHD after observing increased RTV in children with ADHD, and comparable RTV in typically developing controls and children with high functioning autism (HFA) (Johnson, Robertson et al., 2007). In contrast, two

studies reported no significant group differences in RTV between ADHD, HFA, and controls, after controlling for age and IQ (Geurts, Verte, Oosterlaan, Roeyers, & Sergeant, 2004; Raymaekers, Antrop, van der Meere, Wiersema, & Roeyers, 2007). A further two studies included a comorbid (ADHD+ASD) group. One study found that RTV could not differentiate adults with ADHD, ASD, or ADHD+ASD (Nyden et al., 2010). However, in the largest comparative study to date and employing three statistical approaches to index RTV, significantly increased RTV was specific to children with HFA and ADHD+ASD (Geurts et al., 2008). Yet the finding that the ADHD-only and control group displayed comparable performance (Geurts et al., 2008), is inconsistent with the majority of the literature examining RTV in ADHD populations (see section 1.6). Taken together, the evidence suggests that increased RTV is witnessed across both disorders, and is a potentially promising target in the search for common ADHD and ASD genetic susceptibility loci (Rommelse et al., 2011).

Aspects of executive functioning (EF) are also compromised in both disorders, and identified as a promising shared endophenotype to facilitate the hunt for pleiotropic genes in a review of common ASD and ADHD cognitive and brain endophenotypes (Rommelse et al., 2011). As previously mentioned (see section 1.5.1), a large meta-analysis reported response inhibition as one of the strongest and most consistently replicated deficits associated with ADHD (Willcutt et al., 2005). A majority of studies comparing response inhibition across ADHD and ASD groups have employed the go-no/go (GNG) task (also used in this study), with commission errors (CE) as a measure of inhibitory deficits. One study observed impaired response inhibition in children with ADHD, but not in HFA or controls, although the group difference disappeared after controlling for oppositional defiant disorder (ODD) (Raymaekers et al., 2007). In a sample of boys with ADHD, Aspergers Syndrome, and controls, both clinical groups showed impaired inhibition (Nyden, Gillberg, Hjelmquist, & Heiman, 1999). In a study of males aged eight to 16, with ADHD, ASD, and controls groups matched for IQ, the ADHD group displayed significantly more CE (Happe, Booth, Charlton, & Hughes, 2006). When the groups were stratified by age (cut-off specified at age 11), significant improvements in CE were observed in the older cohort of the control and ASD group, compared to their younger counterparts (Happe et al., 2006). In contrast, the older ADHD group did not demonstrate developmental improvements, underlining a distinct response inhibition trajectory specific to ADHD (Happe et al., 2006). In two studies that included single- and dual-disorder groups, the ADHD group exhibited

significantly greater inhibitory deficits (Buhler, Bachmann, Goyert, Heinzel-Gutenbrunner, & Kamp-Becker, 2011; Sinzig, Morsch, Bruning, Schmidt, & Lehmkuhl, 2008). Moreover the combined group had significantly greater deficient response inhibition versus the ASD-only group (Buhler et al., 2011; Sinzig et al., 2008). Overall, the findings are mixed regarding the presence of response inhibitory deficits in pure ASD, although there is suggestive evidence that those children with dual-disorders show deficient response inhibition in line with the ADHD group (but to a lesser degree).

In chapter 3 using a population-based twin sample we showed that RTV and CE display distinct genetic relationships to the two ADHD behavioural symptom domains. Specifically, we found that the RTV-ADHD association largely reflected shared genetic effects between RTV and inattention ($r_G = 0.64$), with a less strong genetic overlap observed with hyperactivity-impulsivity ($r_G = 0.31$). In contrast, CE showed less differentiation between the ADHD symptom domains, although the genetic correlations were overall low ($r_G = 0.11$ and $r_G = 0.17$ for inattention and hyperactivity-impulsivity, respectively). Given these findings and the genetic heterogeneity of ALTs (Dworzynski et al., 2009; Ronald, Happe, Bolton et al., 2006; Ronald et al., 2005; Ronald, Happe, Price et al., 2006), we aimed to investigate if the phenotypic and genetic covariation between ADHD behaviours and ALTs are driven by specific symptom subscales, and if cognitive impairments underlie the observed co-occurrence between ADHD behaviours and ALTs. Using the same population-based twin sample, our study aimed, specifically, to investigate: (1) to what extent are social and non-social ALTs phenotypically and genetically associated with the two ADHD symptom domains of inattention and hyperactivity-impulsivity; (2) to what extent are social and non-social ALTs phenotypically and genetically associated with RTV and CE; and (3) for any significant genetic correlations that emerge in (2), estimate the extent to which genetic influences are shared/distinct with those on inattention and/or hyperactivity-impulsivity.

5.3 Methodology

5.3.1 Sample and procedure

Participants are members of the Study of Activity and Impulsivity Levels in children (SAIL), a general population twin sample (see section 3.3.1 for more details on sample and procedure). The final SAIL sample consisted of 1312 individuals.

5.3.2 Measures

5.3.2.1 Behavioural rating scales

ALTs were rated by parents and teachers when children were aged seven via postal questionnaire. The questionnaire was comprised mainly of behaviours that would be observed in the general population, so items relating to rare behaviours were not included (Ronald et al., 2005). The majority of items were derived from DSM-IV autism criteria, and divided according to criteria as measures of social or non-social ALTs (Ronald et al., 2010; Ronald et al., 2005) (see Table 5.1). The six items in the non-social scale assess obsessive and repetitive behaviours, detail-focused behaviours and restricted interests. The 10 items in the social scale assess peer interactions, social insight, non-verbal behaviours, and unusual communication style. Each item was rated as 'not true' (0), 'somewhat true' (1), or 'certainly true' (2). Summed ratings for the social and non-social subscale were available, respectively, for 959 and 961 SAIL participants. (See section 3.3.2.1 for details regarding ADHD behavioural ratings).

5.3.2.2 Wechsler Intelligence Scales for Children Third Edition (WISC-III)

See section 3.3.2.2.

5.3.2.3 The go-no/go task

See section 3.3.2.3 for more details of the go-no/go (GNG) task.

5.3.2.4 The fast task

See section 3.3.2.4 for more details on the 4-choice reaction time (RT) fast task.

5.3.2.5 Selection of variables for analyses

Teacher and parent ratings for inattention ($r = 0.45$) and hyperactivity-impulsivity ($r = 0.40$), were modestly correlated. Inter-rater correlations were lower, but significant, for both social ($r = 0.20$) and non-social ($r = 0.17$) autistic-like subscales. All correlations were significant ($p < 0.001$). To facilitate comparisons to our previous findings using the same sample, rating subscale scores were summed across informants, and reflect behaviour across situational contexts. See section 3.3.2.5 for selection and treatment of cognitive variables.

Table 5.1 Items used to measure autistic-like traits by social and non-social symptom subscales
Social subscale
<ol style="list-style-type: none"> 1. Has unusual eye gaze, facial expression or gestures 2. Considerate of other people's feelings (R) 3. Rather solitary or tends to play alone 4. Has odd style of communication; old-fashioned, formal, or pedantic 5. Generally liked by other children (R) 6. Can take hints and keep secrets, can be discreet (R) 7. Often says things that are embarrassing for others, without realising 8. Gets on better with adults than with other children 9. Is afraid in social situations 10. Has at least one good friend (R)
Non-social subscale
<ol style="list-style-type: none"> 1. Is extremely distressed by changes to routine or familiar arrangements 2. Notices small details others might miss 3. Insists on doing something over and over so that it interferes with day to day life 4. Tends to check that some things are done exactly 'right' 5. Fussy or over-particular 6. Has a strong interest in an unusual topic
<p><i>Note:</i> (R) reflects where score was reversed</p>

5.3.3 Analyses

5.3.3.1 Structural equation modelling

The structural equation modelling program Mx (Neale, Boker et al., 2006) was used. Models were fitted to age- and sex-regressed unstandardised residual summed scores (cognitive variables were additionally regressed for IQ), and where appropriate transformed to approximate a normal distribution (using the optimised minimal skew command 'lnskew0' in STATA (Stata, 2005)). See section 3.3.3.1 for more details about model fit procedures.

5.3.3.2 Univariate genetic analyses

Under the assumptions of the twin method and twin correlations (see section 1.3), the full genetic ACE model were fitted to cognitive data (DZ correlations were around half of MZ correlation). For behavioural ratings MZ correlations were more than twice DZ correlations. In the absence of significant MZ/DZ variance differences ($p > 0.01$), we fitted the full ADE model.

Within the univariate modelling the presence of sex-specific influences on the phenotypes was tested (see section 3.3.3.3 for more details). Using a p-value threshold of 0.01 to control for multiple testing, we found gender-specific scalar effects (gender-specific phenotypic variance) differences for the majority of variables. Therefore in the multivariate modelling male phenotypic variances were pre- and post-multiplied by a scaling factor.

5.3.3.3 Multivariate genetic analyses

Multivariate designs offer greater power by decreasing the rate of false positive (type I) error rates (by reducing multiple testing). Accordingly, as multivariate models have improved power over univariate models (Schmitz et al., 1998), only multivariate parameter estimates are presented (see section 3.3.3.5). Fitting an ADE model in a multivariate model with other phenotypes that best fit an ACE model (i.e. cognitive variables) can be problematic, as we may have insufficient power to distinguish between A and D (Wood et al., 2008). Accordingly, we fitted a model where D was dropped from behavioural ratings. In this model by not distinguishing between additive and dominant genetic effects, the contribution of shared broad-sense genetic influences to the covariation of traits is assessed.

Correlated factor solution of the full Cholesky Decomposition: In the Cholesky, a triangular decomposition is used, to decompose phenotypic variance and covariance into aetiological influences. A triangular decomposition was run and converted to the mathematical equivalent correlated factor solution (Loehlin, 1996) (see Figure 5.1), in which the order of traits is arbitrary (see section 3.3.3.7 for more details).

Three-variable Cholesky Decomposition: Although the ordering of variables in the Cholesky Decomposition may be arbitrary, for computational reasons cognitive measures were assigned as the first variables, to ascertain how much of the aetiological influences shared between ADHD behaviours and ALTs are independent of influences shared with cognitive measures (see 3.3.3.8 for more details). As such, for these analyses we present the reduced (three-variable) Cholesky decomposition (see Figure 5.2).

5.4 Results

Means and standard deviations for behavioural ratings and cognitive data are given in Table 5.2. Given the variance differences between genders, means and standard deviations are presented separately for males and females.

The focus of this paper is on the covariance of social and non-social ALTs each with ADHD behaviours and cognitive variables. Accordingly in Table 5.3 we present maximum likelihood CTCT correlations for social and non-social ALTs separately with each of the remaining variables, and parameter estimates for the specific relationship of social and non-social ALTs with behavioural ADHD ratings and cognitive variables from the correlated factors solution of the full Cholesky Decomposition. (See Table B.1 in Appendix B for maximum likelihood correlations within and across all variables, and Table B.2 in Appendix B and Figure 5.1 for all parameter estimates from the correlated factors solution of the full Cholesky Decomposition).

Table 5.2 Means and standard deviations for behavioural ratings and cognitive measures

	Hyperactivity- Impulsivity^a	Inattention^a	Social ALTs^a	Non-social ALTs^a	RTV^b	CE^c
MZM	11.06 (8.61) (n=222)	12.70 (8.95) (n=222)	6.01 (3.57) (n=187)	5.86 (3.15) (n=188)	619.06 (350.81) (n=237)	116.52 (34.29) (n=243)
MZF	6.74 (5.89) (n=234)	7.79 (6.51) (n=234)	4.53 (2.99) (n=198)	5.58 (2.44) (n=198)	629.94 (364.15) (n=257)	96.42 (31.47) (n=267)
<i>DZM</i>	<i>11.53 (9.64)</i> <i>(n=360)</i>	<i>14.25 (11.14)</i> <i>(n=360)</i>	<i>6.20 (3.79)</i> <i>(n=284)</i>	<i>6.57 (3.30)</i> <i>(n=285)</i>	<i>631.01 (376.52)</i> <i>(n=376)</i>	<i>115.59 (32.90)</i> <i>(n=391)</i>
<i>DZF</i>	<i>7.31 (6.49)</i> <i>(n=343)</i>	<i>9.06 (7.88)</i> <i>(n=343)</i>	<i>5.08 (3.29)</i> <i>(n=290)</i>	<i>5.80 (2.80)</i> <i>(n=290)</i>	<i>628.04 (359.12)</i> <i>(n=377)</i>	<i>95.61 (33.14)</i> <i>(n=389)</i>

Note: ^a Sum of parent and teacher ratings; ^b Sum of unstandardised data scores across baseline condition of the fast task and slow condition of the go/no-go (GNG) task; ^c Sum of percentages of CE across slow and fast condition of the GNG task; Abbreviations- n: number of observations; MZM: monozygotic male; MZF: monozygotic female; DZM: dizygotic male; DZF: dizygotic female; ALTs: autistic like traits; RTV; Reaction time variability; CE: Commission errors; **MZ data in bold typeface**, *DZ data in italic typeface*

Table 5.3 Maximum-likelihood CTCT correlations (constrained correlated model), and aetiological and phenotypic correlations (standardised correlated factors solution of genetic ACE model) for social and non-social ALTs with ADHD behaviours and cognitive measures

	Cross-trait correlations		Aetiological correlations		Phenotypic correlation	Contribution of covariance accounted for aetiological factors ^a	
	Cross-twin (MZ)	Cross-twin (DZ)	r_G	r_E	r_{PH}	r_{PH-G}	r_{PH-E}
Hyperactivity-impulsivity and:							
Social ALTs	0.31	0.14	0.44	-0.03	0.31	0.32*	-0.01*
	(0.23/0.37)	(0.06/0.22)	(0.33/0.55)	(-0.17/0.11)	(0.24/0.37)		
Non-social ALTs	0.13	0.08	0.20	-0.08	0.11	0.14*	-0.03*
	(0.05/0.19)	(-0.002/0.15)	(0.08/0.32)	(-0.21/0.07)	(0.04/0.18)		
Inattention and:							
Social ALTs	0.31	0.13	0.52	0.03	0.33	0.31	0.01
	(0.23/0.35)	(0.04/0.21)	(0.39/0.65)	(-0.12/0.18)	(0.26/0.39)	(96%)	(4%)
Non-social ALTs	-0.01	0.06	0.05	-0.20	-0.06	0.03*	-0.08*
	(-0.09/0.03)	(-0.02/0.10)	(-0.11/0.21)	(-0.34/-0.05)	(-0.13/0.01)		

RTV and:							
Social ALTs	0.16	0.03	0.32	0.06	0.18	0.16	0.02
	(0.08/0.24)	(-0.05/0.10)	(0.15/0.66)	(-0.07/0.19)	(0.11/0.25)	(87%)	(13%)
Non-social	0.01	0.01	-0.01	-0.07	-0.04	-0.01	-0.03
ALTs	(-0.10/0.05)	(-0.07/0.06)	(-0.20/0.21)	(-0.20/0.06)	(-0.11/0.04)	(13%)	(87%)
CE and:							
Social ALTs	0.01	-0.01	0.12	0.04	0.03	0.04	0.01
	(-0.07/0.09)	(-0.08/0.07)	(-0.35/0.37)	(-0.09/0.16)	(-0.05/0.09)	(71%)	(29%)
Non-social	-0.05	0.02	-0.10	-0.10	-0.09	-0.04	-0.05
ALTs	(-0.13/-0.01)	(-0.06/0.08)	(-0.68/0.15)	(-0.22/0.03)	(-0.15/-0.02)	(42%)	(58%)
<p>Note: 95% Confidence Intervals given in parentheses; Significant ($p < 0.05$) estimates in bold typeface; Due to the lack of quantitative and qualitative sex differences, MZ and DZ correlations are not presented by sex; ^a The contribution of aetiological factors to the phenotypic correlation is given as a raw estimate, and as a percentage in brackets. * It was not possible to formally estimate these proportions, due to the presence of both positive and negative aetiological correlations between relevant variables. Abbreviations- CTCT: cross-twin cross-trait; ALTs: Autistic-like traits; RTV; Reaction time variability; CE: Commission errors; MZ: Monozygotic; DZ: Dizygotic; r_G: broad-sense genetic correlation; r_E: individual-specific environmental correlation; r_{PH}: phenotypic correlation; r_{PH-G}: phenotypic covariance due to broad-sense genetic effects; r_{PH-E}: phenotypic covariance due to individual-specific environmental effects;</p>							

5.4.1 The phenotypic and genetic associations with social and non-social ALTs and the two ADHD symptom domains of inattention and hyperactivity-impulsivity

Social ALTs correlated moderately and equally with both inattention (0.33) and hyperactivity-impulsivity (0.31). In contrast, the association between non-social ALTs and hyperactivity-impulsivity (0.11) was significantly lower. The phenotypic covariance between social ALTs and inattention was predominantly accounted for by shared broad-sense genetic effects (96%). Although the proportion of the phenotypic covariance between hyperactivity-impulsivity and either ALT subscale could not be quantified (aetiological correlations had both positive and negative values), visual inspection of raw estimates (see Table 5.3) suggest that the majority of the covariation was due to shared genes.

The broad-sense genetic correlations for social ALTs with either ADHD behavioural dimension were substantial and showed little differentiation (inattention (0.52) and hyperactivity-impulsivity (0.44)). In contrast, the broad-sense genetic overlap between non-social ALTs and hyperactivity-impulsivity was significantly lower (0.20).

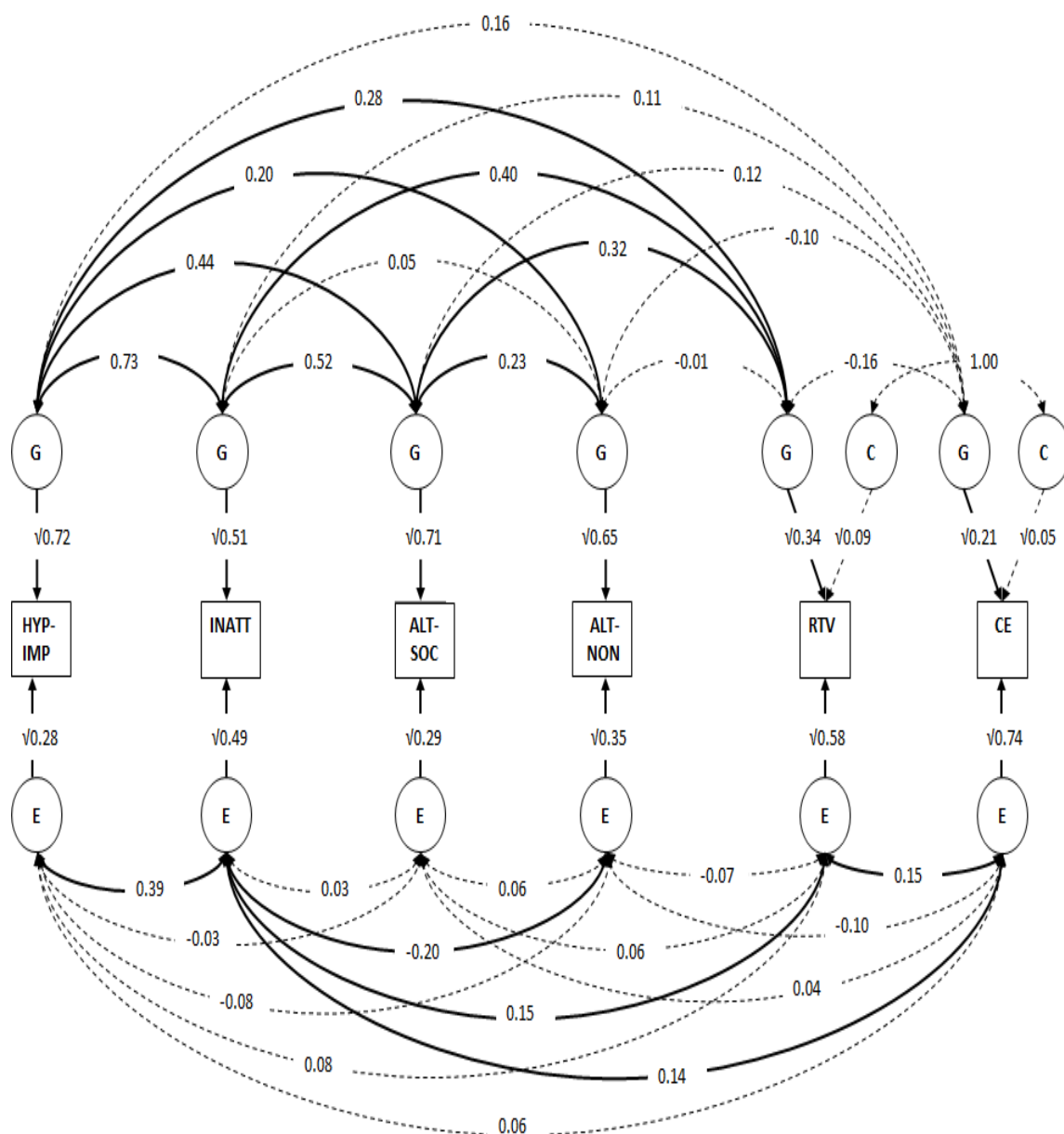
The broad-sense genetic correlation between non-social ALTs and inattention was low and non-significant (0.05), although a significant non-shared environmental correlation was observed (-0.20).

5.4.2 The phenotypic and genetic associations with social and non-social ALTs and RTV and CE

Social ALTs were significantly correlated with RTV (0.18), and showed moderate broad-sense genetic correlations (0.32). Broad-sense genetic effects accounted for the vast majority of the phenotypic covariation (87%) between RTV and social ALTs. Non-social ALTs were significantly negatively correlated with CE (although this was low (-0.09)). An almost equal proportion of genetic (42%) and individual-specific environmental (58%) factors accounted for the phenotypic covariation between CE and non-social ALTs.

All other aetiological correlations between cognitive variables and ALTs were low (-0.10 to 0.12) and non-significant.

Figure 5.1 Correlated factors solution of the full ACE Cholesky Decomposition



Note: Significant parameters are indicated with solid lines; non-significant parameters in dotted lines; Abbreviations- HYP-IMP: Hyperactivity-impulsivity; INATT: Inattention; ALT-SOC: Social autistic-like trait subscale; ALT-NON: Non-social autistic-like trait subscale; RTV: Reaction time variability; CE: Commission errors; G: broad-sense genetic influences; E: individual-specific environmental influences; Model presented for one twin only for ease of presentation;

5.4.3 Aetiological association between social ALTs and inattention, independent of RTV

In the full correlated factors solution (see Figure 5.1), inattention and social ALTs showed strong overlapping broad-sense genetic effects (0.52). Moreover, RTV has substantial shared broad-sense genetic influences with both inattention (0.42) and social ALTs (0.32). Therefore we selected to test in the Cholesky Decomposition (see Figure 5.2), how much of the broad-sense genetic association shared between inattention and social ALTs were due to genetic effects shared with RTV. This was tested using the three-factor Cholesky Decomposition, with RTV assigned as the first variable, and estimated by summing the product of Cholesky genetic paths that are shared with RTV and taking them as a percentage of the total genetic covariance between inattention and social ALTs. We also estimated how much of the individual-specific environmental factors shared between social ALTs and inattention were shared with RTV. (C did not contribute to the covariation between RTV and either inattention or social ALTs).

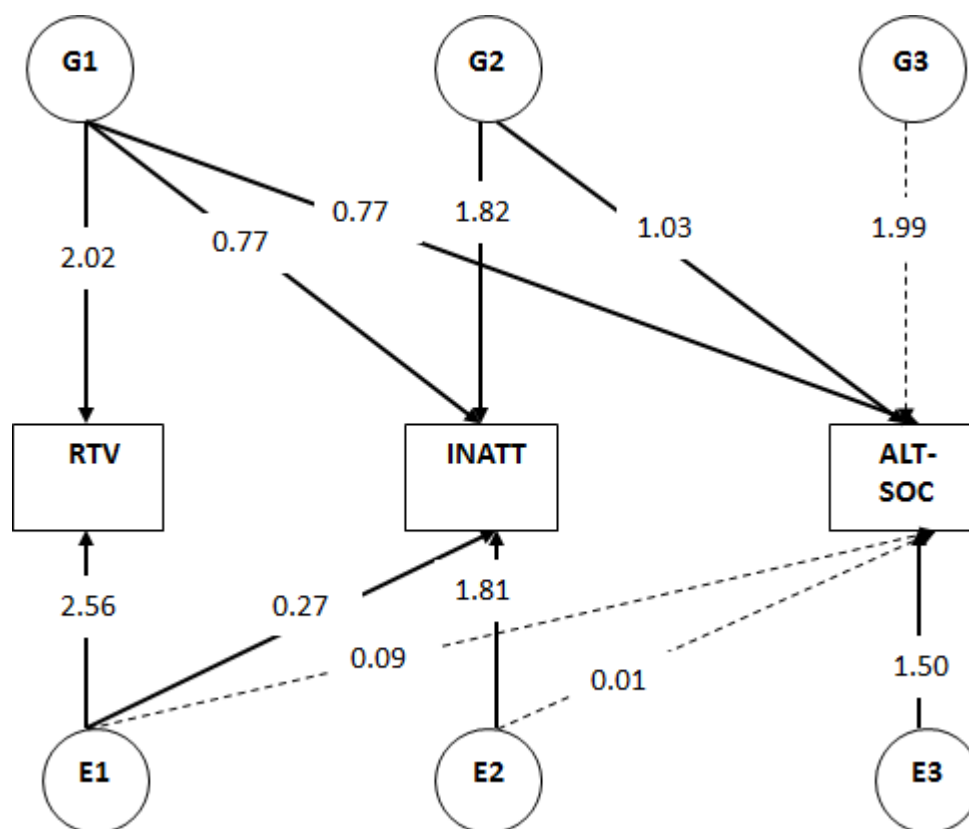
Using the parameter estimates from the Cholesky Decomposition (see Figure 5.2), we estimated that 24% of the broad-sense genetic covariance between inattention and social ALTs was shared with genetic effects underlying RTV:

$$((0.77*0.77) / (0.77*0.77) + (1.82*1.03) = 0.59 / (0.59+1.87) = 0.59/2.47 = 0.24).$$

Therefore the majority (76%) of genetic covariance between social ALTs and inattention occurred independently of genetic effects underlying RTV.

In a similar vein, 43% of the individual-specific environmental covariance between social ALTs and inattention was not shared with RTV.

Figure 5.2 Broad-sense genetic and individual-specific environmental parameter estimates from the three-variable Cholesky Decomposition



Note: unstandardised parameter estimates; significant parameters are indicated with solid lines; non-significant parameters in dotted lines; Abbreviations- RTV: Reaction time variability; INATT: Inattention; ALT-SOC: Social autistic-like traits; Model presented for one twin only for ease of presentation and for broad-sense genetic (G) and individual-specific environmental (E) influences only (the only aetiological factors that contributed to covariation between either social autistic-like traits and inattention with RTV).

5.5 Discussion

This is the first study to examine the covariation between ADHD behaviours and ALTs when both are separated into symptom subscales. We further investigated the phenotypic and genetic associations of two cognitive impairments with both social and non-social ALTs and tested whether shared genetic effects between ADHD and ALT subscales, are shared or distinct with genetic effects for cognitive impairment factors.

Our main finding suggests that the previously observed phenotypic association between ADHD behaviours and ALTs is predominantly driven by social ALTs, equally with both inattention and hyperactivity-impulsivity. There was modest broad-sense genetic overlap between non-social ALTs and hyperactivity-impulsivity ($r_G = 0.20$), whereas broad-sense genetic correlations between social ALTs and both ADHD behavioural dimensions were significantly larger (0.44 to 0.52). Therefore, the previously observed genetic overlap between ADHD behaviours and ALTs is largely driven by social ALTs, equally with both inattention and hyperactivity-impulsivity. These novel findings extend previous studies that have not taken into account the genetic heterogeneity of both ADHD behaviours and ALTs. The only previous study to take into account the genetic heterogeneity of ALT subscales found no differentiation between social and non-social ALTs and ADHD (total) behaviours, in preschool children (Ronald, Edelson et al., 2010). However, we find that when ADHD behaviours are distinguished by their symptom subscales in middle childhood there is a differentiation between social and non-social ALTs.

Previous twin studies have yielded high estimates of shared heritability between ADHD and ALTs, suggesting that future ADHD candidate association studies may benefit from selecting markers associated with ALTs, and vice-versa (Ronald et al., 2008). However, our findings underline that when searching for overlapping genes, it is important to consider the genetic heterogeneity within (and across) ADHD behaviours and ALT symptom subscales. The substantially larger genetic correlation between social ALTs and both inattention and hyperactivity-impulsivity, versus non-social ALTs and hyperactivity-impulsivity, suggest that molecular genetic investigations searching for pleiotropic genes should focus on targeting social ALTs. Targeting non-social ALTs, will likely contribute to the detection of trait-specific genetic risk markers. However, it is important to note that as this is the first study to examine

the aetiological overlap between both ADHD and autistic-like symptom subscales, further studies are needed to confirm these findings.

Second, we observed a significant phenotypic correlation between RTV and social ALTs. This is in line with previous studies suggesting that increased RTV is not specific to ADHD, but also observed in ASDs (see section 5.1). However our findings go beyond this, underlining that the association with RTV and ASDs is driven by social ALTs. This suggests that RTV may potentially discriminate children within the autism spectrum, as children with significant social impairments are likely to manifest increased RTV. However, a previous study reported no significant RTV group differences between children with diagnosed Aspergers Syndrome, HFA, and PDD-NOS (Verte, Geurts, Roeyers, Oosterlaan, & Sergeant, 2006). In chapter 3, RTV was highlighted as a cognitive endophenotype candidate for ADHD, showing significant phenotypic and genetic covariation, particularly with inattention. In this study we extend these previous findings using the same population-based sample, and find evidence to further suggest that RTV may be an endophenotypic marker for social ALTs. Therefore, RTV is an endophenotype shared between ADHD and ASD, but predominantly linked to inattentive behaviours and social impairments. Further work is needed to identify common and unique endophenotypes and pathways within and across disorders (Banaschewski et al., 2005), and should take into account the clinical heterogeneity of ADHD and ASD presentation.

Further analyses suggested that only a minority of the genetic covariation between inattention and social ALTs (24%) was due to shared genetic effects with RTV. Therefore the genetic association between these two behavioural symptoms was largely independent of RTV.

A fourth key finding was that CE showed differential phenotypic covariations with ADHD behaviours versus ALTs. Although phenotypic correlations were low, there was a positive association between CE and both ADHD symptom domains, but a significant inverse relationship with non-social ALTs. This suggests that CE may represent a distinct phenotypic profile across these behaviours, and may provide a means to differentiate between the two disorders, as there was also no significant correlation with social ALTs.

A limitation of our study was that behavioural ratings were not collected simultaneously. The mean age of twins when ALT ratings were completed was 7.06 (sd = 0.28) for parental ratings and 7.32 (sd = 0.23) for teacher ratings. In addition, ALT parental ratings were collected, on average, 18 months earlier than ADHD ratings and cognitive testing, and ALT teacher ratings on average 15 months earlier. Moreover, to keep in line with our previous analysis, parent and teacher ratings were combined (reflective of pervasive rather than situational behaviours), although the correlations between informant ratings for ALTs were modest. However, previous studies examining the phenotypic and aetiological overlap of ADHD behaviours and ALTs, suggest that the pattern of findings is consistent across informants (see section 5.2), supporting the use of combined informant ratings.

Strengths of this study include the use of a population-twin sample, as there are biases inherently associated with clinical and selected samples which may distort estimates of the aetiological associations between co-occurring symptoms. Moreover, by utilising quantitative assessments of these behaviours in a population twin sample, we were also able to investigate the two ADHD and two ALT subscales separately. As outlined above this represents a significant gap in the literature, despite the well established substantial genetic heterogeneity within ADHD behaviours, and particularly within ALTs.

In conclusion, the significant phenotypic and genetic correlations between social ALTs and both ADHD behavioural dimensions largely underlie the previously observed ADHD-ASD covariation. We also found significant phenotypic and genetic covariation between social ALTs and RTV. Despite the substantial genetic overlap between RTV and inattention on the one hand, and RTV and social ALTs on the other hand, the shared genetic effects between social ALTs and inattention are largely independent of genetic effects shared with RTV. Further studies in independent samples are needed to confirm our findings. However, these novel findings can inform the selection of targets for molecular genetic research for overlapping genetic variants. Furthermore, our findings highlight that adopting a multidisciplinary approach by combining cognitive-experimental measures and behavioural ratings in genetically sensitive designs, and separating symptom subscales, may help clarify the link between these two distinct but overlapping disorders.

CHAPTER 6 ADHD AND ATYPICAL HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS FUNCTIONING: EVIDENCE FOR FAMILIAL OVERLAP AND MODERATING EFFECTS OF OPPOSITIONAL BEHAVIOURS

6.1 Abstract

Attention-deficit/hyperactivity disorder (ADHD) has been linked to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, indexed by salivary cortisol. Whether this association is due to overlapping genetic effects or explained by co-occurring oppositional symptoms, remains controversial. We aimed to investigate the phenotypic and aetiological association of ADHD symptoms with cortisol reactivity and diurnal variation, and the moderating effects of co-occurring symptoms. 68 male twin pairs aged 12-15 were selected based on consistently high or low parental ADHD ratings. We obtained salivary cortisol across three time points during a cognitive-electroencephalography (EEG) session and collected on a separate day at three additional time points (awakening, 30 minutes post-awakening and at bedtime) to capture diurnal variation. Growth curve modelling (GCM) was applied to the laboratory measures to examine the association of latent intercept and slope factors and ADHD affection status, and the moderating effects of commonly co-occurring behaviours. We further tested the aetiological overlap between the slope growth curve factor scores and oppositional behaviours, and how much of these shared aetiological influences were independent of aetiological factors underlying ADHD affection status. There were no significant phenotypic associations between individual cortisol reactivity and diurnal variation measures and ADHD affection status. Using GCM, ADHD affection status had a significant main effect on the slope mean, with cortisol levels dropping faster for the ADHD group. A similar result was observed for oppositional behaviours on the slope mean. Further analysis suggested that the association between the slope and ADHD affection status was driven by oppositional behaviours. No main or interactive effects were found when anxiety symptom ratings were modelled with ADHD affection status. Twin modelling of individual slope scores suggested a familial component of covariance with oppositional behaviours; however, we had insufficient power to decompose this familial correlation. In conclusion, we identified the rate of change in cortisol reactivity as the aspect of HPA axis functioning that shows a significant association with ADHD. Further analyses showed that it is likely that this association is primarily driven by oppositional

behaviours, and that this association is explained by familial effects. The study of the pathophysiological processes involved in ADHD may facilitate preventative and treatment interventions in the management of ADHD.

6.2 Introduction

Attention deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterised by age-inappropriate levels of hyperactivity, impulsivity and inattention. ADHD symptoms have a strong genetic component, with heritability estimated at around 70% (Biederman & Faraone, 2005; Burt, 2009). The investigation of objectively-measured physiological and neurobiological measures as potential intermediate ADHD phenotypes may capture underlying processes that lie closer to ADHD genetic liability and delineate pathways from genes to ADHD behaviours.

Components of atypical hypothalamic-pituitary-adrenal (HPA) axis functioning, indexed by cortisol, are associated with ADHD and may reflect a potential intermediate phenotype. Studies on children with diagnosed ADHD have reported deviations in diurnal cortisol variation (Blomqvist et al., 2007; Kaneko, Hoshino, Hashimoto, Okano, & Kumashiro, 1993), low basal cortisol output (Ma, Chen, Chen, Liu, & Wang, 2011), atypical cortisol non-suppression to dexamethosone (Kaneko et al., 1993), and blunted cortisol stress reactivity (McCarthy et al., 2011). Yet other studies have reported greater cortisol reactivity in children diagnosed with ADHD (White & Mulligan, 2005), or a positive correlation (though limited to males) between cortisol productivity and ADHD symptoms in a population-based sample (Hatzinger et al., 2007)). Possible heterogeneity in HPA functioning within ADHD may account for some of these inconsistent findings (Stadler et al., 2011; Yang et al., 2007).

Studies comparing HPA axis activity across children with predominantly inattentive (ADHD-PI) subtype, predominantly hyperactivity-impulsivity (ADHD-PHI) subtype, and ADHD combined (ADHD-C) subtype, report significantly lower baseline cortisol (Ma et al., 2011) and blunted reactivity (Maldonado, Trianes, Cortes, Moreno, & Escobar, 2009) in children with ADHD-PHI subtype. In contrast, one population-based study found an association with severe, but not moderate, inattentive symptoms and blunted cortisol reactivity (Randazzo, Dockray, & Susman,

2008). Moreover individuals were excluded if they endorsed more than two DSM-IV based hyperactivity-impulsivity symptoms, underlining that the observed association with inattentive symptoms was not driven by hyperactivity-impulsivity symptoms (Randazzo et al., 2008).

The samples in the three previously mentioned studies (Ma et al., 2011; Maldonado et al., 2009; Randazzo et al., 2008) were screened for oppositional defiant disorder (ODD) and conduct disorder (CD), and the exclusion of individuals with these co-occurring disorders suggested that such co-occurring symptoms are not confounding ADHD associations. However, the sample sizes in two of these studies were particularly small: ($n = 39$) (Randazzo et al., 2008); ($n = 66$) (Maldonado et al., 2009). Although the sample size was larger in the remaining study ($n = 158$) (Ma et al., 2011), the age range of participating individuals was relatively broad (aged six to 14). Studies based on larger samples suggest that co-occurring disorders do moderate the association with HPA dysfunction and ADHD. In a large sample of over 200 children, the cortisol awakening response (CAR) (the typical increase in cortisol levels from awakening to 30 minutes later) was compared according to comorbidity in children diagnosed with ADHD, and in typically developing controls (Freitag et al., 2009). No significant differences were found between children with ADHD and comorbid anxiety disorders, children with ADHD without comorbid disorders, or control children (Freitag et al., 2009). However, a blunted CAR was observed in children with ADHD and comorbid ODD, versus controls, ADHD and comorbid CD, and ADHD without co-occurring disorders (Freitag et al., 2009). In a large sample of pre-adolescent males ($n = 170$), in contrast with the above findings, hyper cortisol reactivity was observed in individuals with comorbid anxiety disorders (Hastings, Fortier, Utendale, Simard, & Robaey, 2009). Partially in line with the above findings, attenuated cortisol reactivity was associated with comorbid disruptive behaviour disorders (DBD), although only in those with ADHD-PI or ADHD-PHI subtypes (Hastings et al., 2009). However, reactivity in this study was based on only two measures (pre and post-stressor). In a large study of 95 children with sampling collected over seven points, no baseline differences in cortisol were observed (Snoek, Van Goozen, Matthys, Buitelaar, & van Engeland, 2004). However compared to typically developing controls or children with ADHD and no comorbidity, significantly weaker cortisol reactivity was observed in children with ADHD+ODD and ODD alone (Snoek et al., 2004). These findings highlight that the covariation between ADHD and HPA-axis dysfunctioning is complicated by comorbidity.

The heritability of HPA axis functioning has been investigated in twin studies. A review reported substantial variation in heritability estimates for basal cortisol measures (0-84%; (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003)), likely reflecting varying methodologies. A meta-analysis on five comparable studies yielded a heritability estimate of 62% for basal cortisol (Bartels et al., 2003). A recent study, based on a subsample of same-sex twins selected from the Twins' Early Development Study (TEDS), which this sample is also derived from, found substantial heritability for single-point measures (around 60%) and more moderate heritability for cortisol reactivity (44%) to a computerised behavioural challenge (Steptoe, van Jaarsveld, Semmler, Plomin, & Wardle, 2009). The extent to which the genetic influences on cortisol measures are shared with genetic influences on ADHD has not yet been addressed, and is a key objective of this study.

This study aimed to investigate the association between ADHD affection status and both cortisol reactivity and diurnal variation in a sample of male adolescent twin pairs selected from a population-based sample for high and low ADHD symptoms. Specifically, we aimed to examine: (1) group differences in individual cortisol levels and indices of cortisol activity by ADHD affection status; (2) explore the aetiological overlap between ADHD affection status and indices of cortisol activity; (3) explore the relationship of ADHD affection status and the dynamics of cortisol reactivity captured by growth curve factors from a linear growth curve model (GCM); (4) explore the relationship of growth curve factors with ADHD affection status while modelling potential covariates (sampling conditions and demographic factors); (5) test whether the effect of ADHD affection status on growth curve factors can be explained/moderated by anxiety-shy or oppositional behaviours; and (6) explore the genetic relationship between ADHD affection status, oppositional behaviours and the individual slope scores derived from the GCM. We hypothesised that ADHD affection status would be associated with blunted cortisol reactivity and atypical diurnal variation, and that oppositional behaviours would moderate the effect of ADHD affection status. We had no *a priori* hypotheses regarding the moderating effects of anxiety-shy ratings on cortisol-ADHD associations, or the degree of shared genetic influences between ADHD behaviours and indicators of HPA-axis activity.

6.3 Methodology

6.3.1 Sample and procedure

The sample was selected from TEDS, a birth cohort study which invited all twins born in England and Wales between 1994 and 1996 to enrol, (Trouton et al., 2002). Zygosity was determined using a zygosity questionnaire that has been shown to have 95% accuracy (Price et al., 2000). Where zygosity was unclear from this questionnaire, DNA testing was conducted. The TEDS sample is representative of the general population in terms of parental education, ethnicity and employment status (Oliver & Plomin, 2007).

The NEurophysiological study of Activity and Attention in Twins (NEAAT) subset used in this study, consisted of 68 male twin pairs aged between 12 and 15. Twin pairs were selected based on latent class trajectory analysis of ADHD symptoms at ages eight, 12 and 14, using the 18 DSM-IV based ADHD items from the Conners' Parent Rating Scale (Conners et al., 1998a) (see Appendix C for supplementary information on the selection analysis). This approach identified sub-groups of individuals who have been consistently rated by parents as having high or low ADHD symptoms and thus ensured selection of twin pairs consistently concordant or discordant for high levels of ADHD symptoms (corresponding to a clinical diagnosis) or unaffected controls (consistently low ADHD symptom ratings). The selected sample consisted of 22 pairs concordant for high levels of ADHD symptoms (monozygotic (MZ): 11; dizygotic (DZ): 11), eight pairs discordant for ADHD symptoms (MZ: 2; DZ: 6) and 38 control pairs concordant for low levels of ADHD symptoms (MZ: 22; DZ: 16). When subdividing our sample according to ADHD symptom ratings, 84 participants were classified as controls (low ADHD symptom scores) and 52 participants classified in the ADHD group (high ADHD symptom score).

Participating families gave their written informed consent and the study was approved by King's College London Psychiatry, Nursing and Midwifery Research Ethics Sub-Committee (PNM/08/09-089).

6.3.2 Measures

6.3.2.1 Behavioural rating scales

Parents completed the Long Version of the Conners' Parent Revised Rating Scale (CPRS-R: L (Conners et al., 1998a)). Items were used from the 18-item DSM-IV based ADHD subscale, the eight-item anxiety-shy subscale, and the 10-item oppositionality (OPP) subscale. The items in the OPP subscale largely correspond with DSM-IV criteria for Oppositional Defiant Disorder (ODD), although one item ('fights') is more directly related to symptoms of Conduct Disorder (CD). Accordingly the term ODD is avoided, and the behaviour labelled as oppositional after the name of the subscale, which largely reflects ODD symptoms in the general population.

6.3.2.2 Salivary cortisol

Salivary cortisol was obtained using the passive drool method (expressing saliva through a short straw into a small plastic vial). No saliva flow stimulant was used. Saliva was sampled at three points during the cognitive-electroencephalography (EEG) session. The baseline measure (Lab_1) was collected after participants watched a cartoon for half an hour and an additional resting period of five minutes. Following this, the child completed a number of cognitive tasks, with continuous EEG measurement, which required concentration and were repetitive in nature. On average, cortisol levels peak 20 minutes after stressor onset (Hirvikoski et al., 2009). Therefore in order to index participants' response to the second cognitive task, saliva was next obtained at the end of the last cognitive task (Lab_2; approximately an hour after baseline measurement). The final salivary sample (Lab_3) was collected approximately 20 minutes later to index response to the third cognitive task.

At the end of testing, participants were asked if they were willing to provide an additional three saliva samples at home. If participants agreed, families were provided with detailed oral and written instructions for sampling procedures. Participants were asked to collect a sample immediately upon awakening (Home_1), 30 minutes post-awakening (Home_2), and at bedtime (Home_3)), and instructed to record awakening time and sampling times on a supplementary form, so that compliance could be monitored. Participants were requested to refrain from eating, drinking and brushing teeth (to avoid abrasion and micro-vascular leakage) before the first two home samples were collected. As a consequence of such constraints potentially interfering with school routines, sampling took place at weekends or on holidays.

Other than these requests for compliance, participants were free to follow their normal routine. Samples were stored in home refrigerators until returned by mail to the lab.

11 individuals were unable or did not agree to participate in saliva collection during the laboratory testing session, and an additional 33 participants did not provide saliva samples from home. There were no significant differences in age, behavioural measures or ADHD group status between those who did or did not participate. Reported sampling times for morning samples were checked for adherence to sampling procedure, with all samples collected within 20 minutes (mean = three minutes) of designated time (awakening, and 30 minutes post-awakening).

All uncentrifuged saliva samples were stored in a -80°C medical freezer, until completion of the study when they were assayed for cortisol. Salivary samples from 10 participants were sent for preliminary analysis, with all remaining samples analysed in a single batch using a high sensitivity chemiluminescence assay (Salimetrics, Cambridge, UK). The lower limit of detectable sensitivity was $< 0.003 \mu\text{g/dL}$ (micrograms per decilitre). Coefficients of variance were below 10%, and therefore not subjected to re-testing. Cortisol concentrations were provided in $\mu\text{g/dL}$ and converted into nmol/l (nanomoles per litre) by multiplying original values by 27.59.

A total of 375 laboratory and 276 home samples were sent for analysis. One laboratory sample had insufficient saliva volume to test for cortisol, and 34 home samples were unable to have cortisol levels determined (11 had insufficient volume to test for cortisol; 20 samples had too low a limit of cortisol to detect; and three samples lost their identification label during transit).

Cortisol values were screened for extreme values. All laboratory and home samples from one control participant were higher than three standard deviations from the mean, and designated as missing. An extreme value for the second point of sampling in the laboratory for another control participant was designated with the highest individual corresponding value within three standard deviations from the mean value. In total we had 371 valid concentration values obtained from the laboratory (Lab_1 ($n = 124$); Lab_2 ($n = 124$); and Lab_3 ($n = 123$)), and 149 valid samples obtained from home (Home_1 ($n = 90$); Home_2 ($n = 90$); Home_3 ($n = 59$)), that were included in analysis.

On the day of laboratory testing, four children had taken over-the-counter medication (e.g. pain killers) and an additional five children had taken steroid-based asthma inhalers. Two dichotomous variables were created (steroid-based medication (yes/no), and other medication (yes/no)). There were no significant differences according to medication status for either variable, and therefore all participants' samples were retained for analysis.

To assess cortisol changes during the cognitive-EEG session, reactivity ratio change scores between samples were computed, taking into account the influence of the initial value on the magnitude of cortisol change between sampling points (e.g. dividing the difference by the initial value). In addition, two area-under-the-curve (AUC) indices of cortisol output were calculated (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003): AUC with respect to ground (AUC_g; reflecting total cortisol secretion) and AUC with respect to increase (AUC_i; capturing changes in cortisol output over time). Four parameters capturing aspects of diurnal variation were calculated based on home sampling. With respect to the CAR, the delta (absolute) change of the two morning values was generated. Cortisol output throughout the day was calculated using the AUC_g, AUC_i, and the slope of the diurnal rhythm (reflecting the decline in cortisol levels per hour, by subtracting the bedtime from awakening value, divided by the number of hours between sampling points).

6.3.3 Analyses

6.3.3.1 *Group mean differences*

Group mean differences for demographic factors (age and behavioural ratings)), sampling conditions (season of sampling, sampling times, and awakening times), and individual cortisol concentration levels and indices of cortisol activity, were analysed using the regress command in STATA (Stata, 2005), which corrects for non-independent observations (e.g. twin pairs) by using a robust cluster command to estimate standard errors. Means stratified according to ADHD affection status (high or low ADHD symptoms ratings) are summarised in Table 6.1.

6.3.3.2 *Genetic model fitting analysis*

6.3.3.2.1 *Relationship of ADHD affection status and indices of cortisol activity*

Data preparation: To limit the number of variables tested, only composite measures of cortisol were taken forward for genetic modelling (for cognitive-EEG session: ratio change between sampling points, AUCg and AUCi; for home sampling: CAR, AUCg, AUCi and diurnal slope). As all these measures were positively skewed, transformations were applied to the data using the optimised minimal skew through the 'lnskew0' command in STATA (Stata, 2005). As simultaneous analyses of dichotomous traits (ADHD affection status) and continuous traits (cortisol) cannot be performed in the structural equation modelling (SEM) program Mx (Neale, Boker et al., 2006), each cortisol variable was ordinalized into five equal classes (in terms of proportions), thereby capturing most of the information of continuous data.

Twin correlations: Threshold liability modelling was performed to test a model in which the correlational structure of the data was constrained to generate: i) MZ and (ii) DZ cross-twin within-trait (CTWT) correlations for cortisol measures; (iii) MZ and (iv) DZ cross-twin cross-trait (CTCT) correlations (e.g. comparing twin 1's ADHD affection status with co-twin's cortisol value); and (v) a phenotypic correlation between ADHD affection status and cortisol measure. In addition, the selected nature of the sample required an ascertainment correction, which can be omitted by simply fixing the model parameters of the selection variable (ADHD). This means fixing the MZ and DZ cross-twin correlations for ADHD to point estimates derived from the mean heritability pooled across over 20 twin studies ($h^2 = 0.76$; $r_{MZ} = 0.76$, $r_{DZ} = 0.38$) (Faraone et al., 2005), and fixing ADHD prevalence to a lifetime risk of 5% (Polanczyk et al., 2007). This model has been successfully applied to schizophrenia and bipolar disorder in analyses on brain volumes (Rijsdijk et al., 2005) and neuropsychological measures (Toulopoulou et al., 2007), as well as on electrophysiological parameters on the same sample as reported here (Tye, McLoughlin, Kuntsi, & Asherson). Since there were no significant phenotypic or cross-twin cross-trait correlations between ADHD affection status and any of the cortisol variables (Table 6.2), we did not conduct genetic and environmental decomposition of these correlations.

6.3.3.2.2 Genetic Growth Curve Models (GCM)

Genetic GCM testing main effect of ADHD affection status on intercept and slope factors: To capture the dynamics of cortisol activity across the cognitive-EEG session as an index of HPA

axis function (Figure 6.2), a linear GCM was fitted to describe the data by means of two latent factors: the intercept (or baseline (I)) and the slope (or rate of change (S)). The loadings for the intercept are fixed at 1, and those of the slope to 0, 1 and 2 to reflect the linear trajectory of cortisol over the testing period. GCM captures each individual's trajectory across all sampling points, rather than focussing on individual measures (Hagger-Johnson, Whiteman, Wawrzyniak, & Holroyd, 2010). Another advantages of GCM is the non-requirement of complete data for every participant and estimating both parameters simultaneously within the same model (Hagger-Johnson et al., 2010). (Note that a GCM was not fitted to saliva sampled at home, as typical diurnal variation follows a curvature trajectory, which requires at least four samples for all necessary factors to be identified).

Due to the genetic nature of the twin sample, the aetiological variance and covariance of the latent intercept and slope factors can be estimated according to general genetic theory and maximum likelihood model fitting estimation (Neale & McArdle, 2000). In brief, twin studies enable us to disentangle the extent to which a trait is influenced by additive genetic factors (A), shared environmental factors (C), and non-shared environmental influences (E). This method relies on the difference in genetic relatedness between MZ twins, who share all of their genetic information, and DZ twins who share, on average, only 50% of their additive genes. Multivariate genetic models are able to estimate the underlying variance components of individual variables (or latent factors), and the aetiological components of phenotypic covariance between multiple traits (or factors). The information for this is derived from the MZ:DZ ratio of CTCT correlations.

To assess the relationship between the latent growth curve factors and ADHD affection status, we modelled the main effects of a moderator variable (ADHD group status) on the intercept and slope means. A significant main effect of ADHD affection status (moderator) on growth curve factor means (outcome) implicates the (familial) correlation between ADHD and the intercept and slope latent factors (Purcell, 2002).

Genetic GCM testing main effect of ADHD affection status on growth factors while controlling for covariates and modelling moderating effects of anxiety-shy and oppositional behaviours:

Following this we incorporated sampling conditions and age to assess if there was a main effect on the intercept and slope factor mean, and any interactive effect with ADHD affection status on these means. We then modelled anxiety-shy and oppositional behaviours individually as moderator variables on the intercept and slope mean. If the effect of ADHD affection status on growth curve factors disappeared when an additional moderator is simultaneously modelled, we can infer that the moderator likely drives any observed (familial) associations with ADHD affection status. In a final model (see Figure 6.2), we incorporated all variables which showed a significant effect on the means of the latent growth curve factors.

6.3.3.2.3 Multivariate genetic model of ADHD affection status, oppositional behaviours and derived slope factor scores

Based on the previous series of models we conducted threshold model fitting analysis to directly assess the genetic relationship between individual values of the slope growth curve factor (derived by MPLUS), ADHD affection status and oppositional behaviours. However as previously outlined, Mx does not allow mixing continuous (slope factor scores and oppositional behaviours) and ordinal (ADHD affection status) variables. Accordingly, the slope and oppositional behaviour variables were ordinalized into five equal classes (in terms of proportions), thereby capturing most of the information of continuous data. In addition (as explained above), to deal with the selected nature of the sample we fixed the model parameters of ADHD to known population values: $h^2 = 0.76$, $c^2 = 0$, $e^2 = 0.24$ (Faraone et al., 2005); and the threshold to reflect a prevalence of lifetime risk: 5% (Polanczyk et al., 2007).

Correlated factors solution of the Cholesky Decomposition: A triangular Cholesky Decomposition was run, and converted and interpreted as the mathematical equivalent correlated factors solution (Loehlin, 1996) (see Figure 6.3), where the ordering of measured variables is arbitrary (see section 1.3.1.5 for more details).

Cholesky Decomposition: Using the triangular Cholesky Decomposition (see Figure 6.4), variables can be assigned to a set order *a priori*, to establish how much of the covariation between traits is independent of shared aetiological effects with other traits (see section 1.3.1.4 for more details). Therefore in our model incorporating ADHD affection status,

oppositional behaviours and the slope factor scores, ADHD affection status was assigned as the first variable, to allow the estimation of the extent to which covariance between the slope and oppositional behaviours was independent of shared aetiological influences with ADHD affection status.

6.4 Results

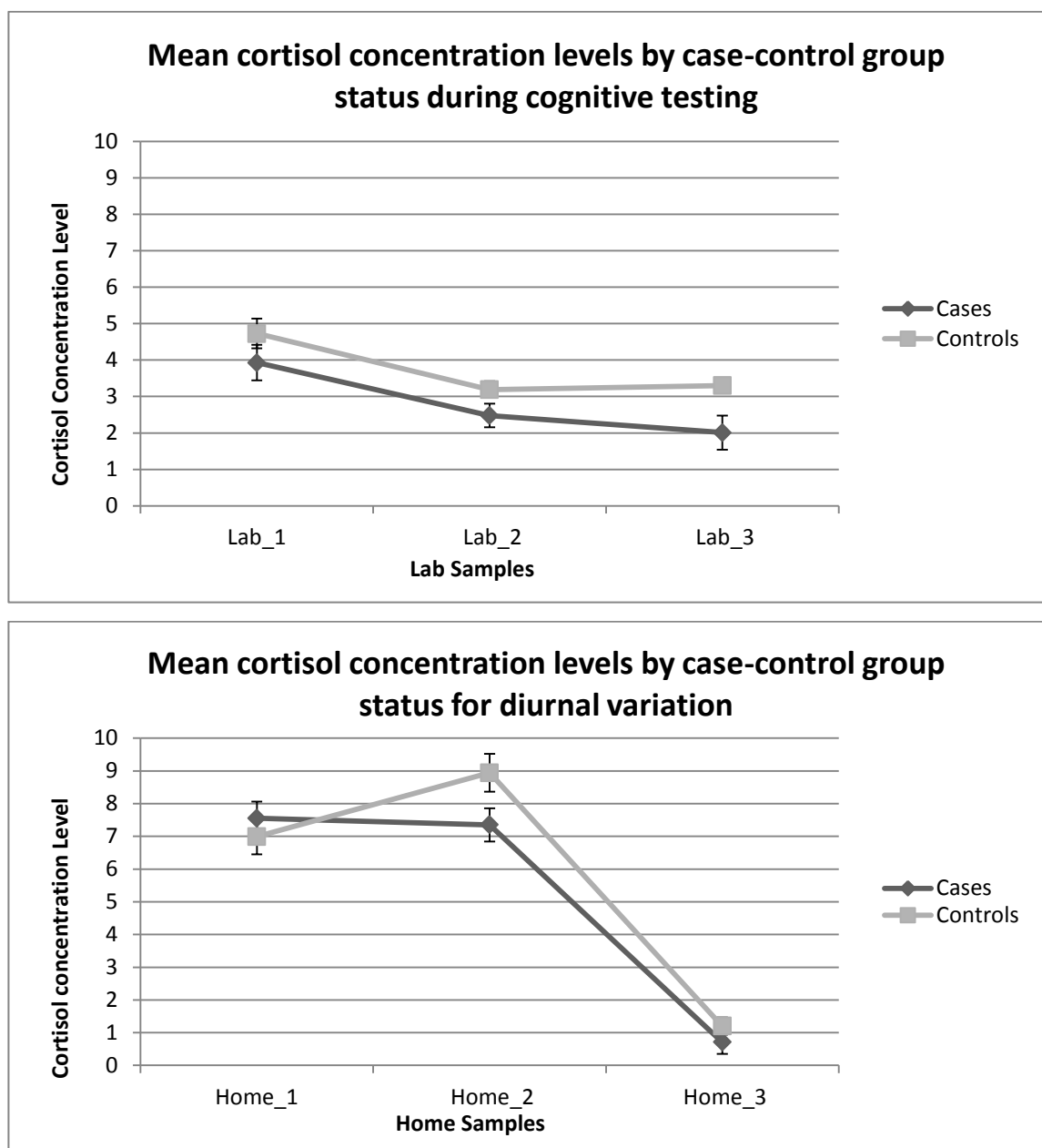
6.4.1 Group mean differences

As expected, the ADHD group had significantly higher mean symptom scores for DSM-IV-based ADHD, confirming that the two groups differed according to key ADHD diagnostic criteria (see Table 6.1). The ADHD group also had significantly higher oppositional behaviour rating scores, but did not significantly differ from controls according to anxiety-shy symptoms. The ADHD group were significantly younger than controls, and were more likely to have participated during months with more light (March to September). In addition, on the day of the cognitive-EEG session, the ADHD group had significantly later awakening and later sampling times. When cortisol was sampled at home, awakening time, sampling time, and non-compliance to sampling protocol (minutes deviated), did not significantly differ between groups. As age and sampling conditions can influence cortisol concentrations, group differences in mean individual cortisol levels and indices of cortisol activity were additionally tested after regressing out sampling conditions and age that displayed group differences.

Mean cortisol levels for both groups declined from baseline levels during cognitive-EEG session (see Figure 6.1 (upper panel) and Table 6.1). Cortisol mean levels at the last sampling point were significantly lower for the ADHD groups versus controls, although this group difference did not retain significance after regressing out potential confounders (age, awakening time, sampling time, and season of sampling) ($t = -1.34$, $p = 0.18$). In addition, when comparing the change between the last two sampling points, cortisol dropped in the ADHD group and increased in controls, and this group difference increased in significance after regressing the confounding effects of age and sampling conditions ($t = -2.58$, $p = 0.01$).

Mean levels for controls for home samples exhibited the typical diurnal variation: high levels at awakening, increasing when sampled 30-minutes later (CAR), and low at bedtime (see Figure 6.1 (lower panel) and Table 6.1). In contrast, the ADHD group did not display a typical CAR, as mean levels were highest at awakening. Accordingly, they had significantly lower cortisol concentrations 30 minutes post-awakening, and a negative CAR. In addition, cortisol productivity throughout the day, as captured by the AUC_G, was significantly lower in the ADHD group. None of these differences remained significant after controlling for age and season of sampling.

Figure 6.1 Mean salivary cortisol concentration by group status during cognitive testing (upper panel) and measuring diurnal variation (lower panel)



Note: Mean raw cortisol concentration values shown in nmol/L during cognitive testing paradigm (upper panel) and measuring diurnal variation (lower panel); Lab_1 was pre-task (baseline) measure; Lab_2 was obtained at end of cognitive testing; Lab_3 was taken at end of entire assessment; Home_1 was taken at awakening; Home_2 was taken 30-minutes post awakening; Home_3 was taken just before bedtime;

Table 6.1 Characteristics of the sample

	ADHD group	Control	T score (p value)
Behavioural ratings			
DSM-IV Total ADHD	57.65 (10.03)	43.14 (4.07)	8.83 (p < 0.001)
Oppositionality	58.33 (14.05)	45.13 (6.51)	5.20 (p < 0.001)
Anxiety	55.15 (12.73)	49.23 (8.33)	3.03 (p = 0.003)
Demographics			
Age	14.00 (0.69)	14.53 (0.90)	-2.88 (p = 0.005)
Sampling Factors			
Season of sampling	36 (69%)	18 (21%)	30.67 (p < 0.001)
Awakening Time- lab testing	8.09 (1.68)	7.36 (1.36)	2.02 (p = 0.05)
Time of Lab_1	14.81 (2.50)	13.58 (2.52)	2.25 (p = 0.03)
Time of Lab_2	15.84 (2.51)	14.60 (2.50)	2.25 (p = 0.03)
Time of Lab_3	16.22 (2.50)	14.99 (2.48)	2.26 (p = 0.03)
Awakening Time- Home sampling	8.97 (1.47)	8.89 (1.22)	0.22 (p = 0.83)
Time of Home_1	9.02 (1.46)	8.95 (1.23)	0.18 (p = 0.86)
Time of Home_2	9.54 (1.45)	9.45 (1.25)	0.24 (p = 0.81)
Time of Home_3	22.63 (1.16)	22.31 (1.16)	0.94 (p = 0.35)
Lab-based cortisol measures			
Lab_1	3.93 (2.87)	4.73 (4.24)	-1.25 (p = 0.22)
Lab_2	2.48 (1.59)	3.18 (2.74)	-1.65 (p = 0.11)
Lab_3	2.01 (1.33)	3.30 (3.93)	-2.49 (p = 0.02)
Ratio change (Lab_1 to Lab_2)	-0.15 (0.59)	0.01 (1.19)	-0.97 (p = 0.34)

Ratio change (Lab_2 to Lab_3)	-0.11 (0.37)	0.22 (1.35)	-2.07 (p = 0.04)
AUCg	4.17 (2.62)	5.42 (4.57)	-1.84 (p = 0.07)
AUCi	-1.37 (1.98)	-1.41(2.83)	-0.10 (p = 0.92)
<i>Home-based cortisol measures</i>			
Home_1	7.55 (3.24)	6.99 (3.72)	0.71 (p = 0.48)
Home_2	7.35 (3.49)	8.94 (3.68)	-1.99 (p = 0.05)
Home_3	0.71 (1.21)	1.20 (2.22)	-1.09 (p = 0.28)
CAR	-0.21 (4.88)	1.98 (4.75)	-2.20 (p = 0.03)
Diurnal Slope	-0.53 (0.28)	-0.45 (0.37)	-0.87 (p = 0.39)
AUCg	57.75 (24.83)	71.99 (29.34)	2.12 (p = 0.04)
AUCi	-46.64 (52.68)	-22.36 (55.89)	-1.61 (p = 0.12)

Note: Data are presented for the ADHD group and controls, as means and standard deviations in parenthesis, unless otherwise stated; Parental behavioural ratings (t-scores) were obtained from the Long Version of the Parent Conners' Rating Scale (Conners et al., 1998a) on the day of testing; For sample times, means and standard deviations shows time as a proportion; Lab_1 was pre-task (baseline) measure; Lab_2 was obtained at end of cognitive testing; Lab_3 was taken at end of entire assessment; Home_1 was taken at awakening; Home_2 was taken 30 minutes post awakening; Home_3 was taken just before bedtime; AUCg (Area under the curve with respect to ground); AUCi (area under the curve with respect to increase); CAR (cortisol awakening response (difference between two morning samples)); diurnal slope calculated by subtracting bedtime from awakening value, divided by number of hours between two samples; raw cortisol levels are shown in nmol/L; group comparison of means values, based on raw data; chi-square test for dichotomous variables; Bold typeface indicates that group mean difference is significant (p < 0.05).

6.4.2 Genetic model fitting analyses

6.4.2.1 Relationship between ADHD affection status and indices of cortisol activity

Table 6.2 presents the CTWT and CTCT correlations for MZ and DZ pairs. When examining the CTWT correlations for cortisol composite measures, the majority of 95% Confidence Intervals (CIs) for MZs and DZs included the value zero, indicating non-significance. A similar pattern was observed for CTCT correlations. As none of the phenotypic correlations with ADHD affection status were significant, formal genetic twin modelling of the bivariate relationship of cortisol phenotypes and ADHD affection was not conducted.

Table 6.2 Cross-twin within-trait correlations for cortisol composite measures; Cross-twin cross-trait correlations for ADHD affection status and cortisol composite measures; Phenotypic correlations between ADHD affection status and cortisol composite measures

	Cortisol		Cross-twin cross-trait correlations (CTCT) ^a		Phenotypic correlation ^a
	rMZ	rDZ	MZ	DZ	
Lab Samples					
Ratio change (Lab_1 to Lab_2)	0.09 (-0.31/0.45)	0.48 (0.10/0.72)	0.13 (-0.23/0.47)	-0.01 (-0.26/0.25)	0.01 (-0.21/0.23)
Ratio change (Lab_2 to Lab_3)	0.18 (-0.27/0.55)	-0.19 (-0.51/0.19)	-0.23 (-0.58/0.10)	-0.27 (-0.50/-0.01)	-0.23 (-0.44/0.02)
AUCg	0.15 (-0.25/0.50)	0.24 (-0.21/0.59)	-0.03 (-0.34/0.27)	0.09 (-0.18/0.35)	-0.11 (-0.31/0.12)
AUCi	-0.07 (-0.42/0.29)	0.42 (-0.004/0.70)	-0.09 (-0.40/0.24)	0.11 (-0.18/0.37)	0.02 (-0.21/0.25)
Home samples					
CAR	-0.05 (-0.51/0.44)	-0.08 (-0.51/0.39)	-0.08 (-0.35/0.32)	0.29 (-0.03/0.55)	0.14(-0.14/0.38)
Diurnal Slope	0.63 (0.07/0.90)	-0.38 (-0.84/0.61)	0.09 (-0.32/0.42)	-0.31 (-0.71/0.10)	-0.17 (-0.43/0.22)
AUCg	0.15 (-0.73/0.60)	0.68 (-0.91/0.14)	-0.01 (-0.37/0.48)	0.20 (-0.23/0.62)	0.19 (-0.22/0.52)
AUCi	0.09 (-0.62/0.71)	-0.52 (-0.84/0.37)	0.12 (-0.43/0.29)	0.42 (-0.01/0.72)	0.19 (-0.15/0.46)

Note: Cortisol composite measures were regressed (for lab-based measures, by age, season, awakening time, and sampling time; for home-based measures by age and season) and normalised. MZ: monozygotic; DZ: dizygotic; Lab_1 was a pre-task (baseline) measure; Lab_2 was obtained at end of cognitive-EEG session; Lab_3 was taken at end of entire assessment; AUCg: Area under the curve with respect to ground; AUCi: area under the curve with respect to increase; CAR: cortisol awakening response (difference between two morning (awakening and 30-minutes post awakening) samples); diurnal slope calculated by subtracting bedtime from awakening value, divided by number of hours between two samples; 95% Confidence Intervals (CIs) in parenthesis (CIs including value 0 are non-significant) CIs not straddling zero are indicated by bold typeface; ^a phenotypic correlations between ADHD affection status and cortisol composite measures;

6.4.2.2 Testing main effects of ADHD affection status on intercept and slope factors

There was no significant main effect of ADHD affection status on the mean of the intercept (-0.001 (95% CIs: $-0.27/0.26$)). In contrast, there was a significant effect of group status on the mean of the slope (-0.16 ($-0.29/-0.03$)), so that the slope mean for controls was estimated at -0.24 , and that for the ADHD group at -0.40 ($-0.24 + -0.16$). The negative value corresponds to the pattern of reactivity over the cognitive-EEG session, implicating that cortisol concentrations decreased for both groups; however, cortisol levels dropped significantly faster for the ADHD group.

6.4.2.3 Testing main effects of ADHD affection status on growth curve factors while controlling for covariates and modelling moderating effects of anxiety-shy and oppositional behaviours

The above results were obtained from non-regressed cortisol variables, as the means of the individual sample variables in a GCM are expressed as a function of the intercept and slope parameters. However, to control for the potentially confounding effects of age, and sampling conditions, the above model was extended by incorporating these as covariates in the model of the means of intercept and slope factors and running a series of models.

The only significant main effect was that increased age was associated with heightened baseline (intercept) values (0.23 ($0.09/0.37$)). In this model, the main effect of ADHD affection status on the slope mean, noted above, remained virtually the same (-0.16 ($-0.30/-0.03$)). When all sampling conditions were incorporated simultaneously, the moderating effect of ADHD affection status on the slope mean, and age on the intercept mean, remained significant.

In a final set of models, anxiety-shy and oppositional behavioural ratings were individually incorporated. Anxiety-shy symptoms did not have a significant main effect on either the intercept or slope factor mean, and there was no interactive effect with ADHD group status on these means. In addition, the main effect of ADHD affection status on the slope mean remained significant. The same pattern of results emerged when age was modelled as a covariate, and in line with the previous model age had a significant main effect on intercept slope.

When oppositional behaviours were modelled with ADHD affection status, there was no significant main effect on the intercept mean. In contrast, there was a significant main effect on the mean of the slope: with the rate of decline dropping faster as oppositional symptoms increase (-0.17 (-0.30/-0.04)). Moreover, the effect of ADHD affection status on the slope was no longer significant (-0.09 (-0.24/0.06)), and there was no significant interaction between oppositional behaviours and ADHD affection status on the slope. There was no change in results when age was included as a covariate (see Figure 6.2 for final model), which similarly showed a significant main effect on the intercept mean.

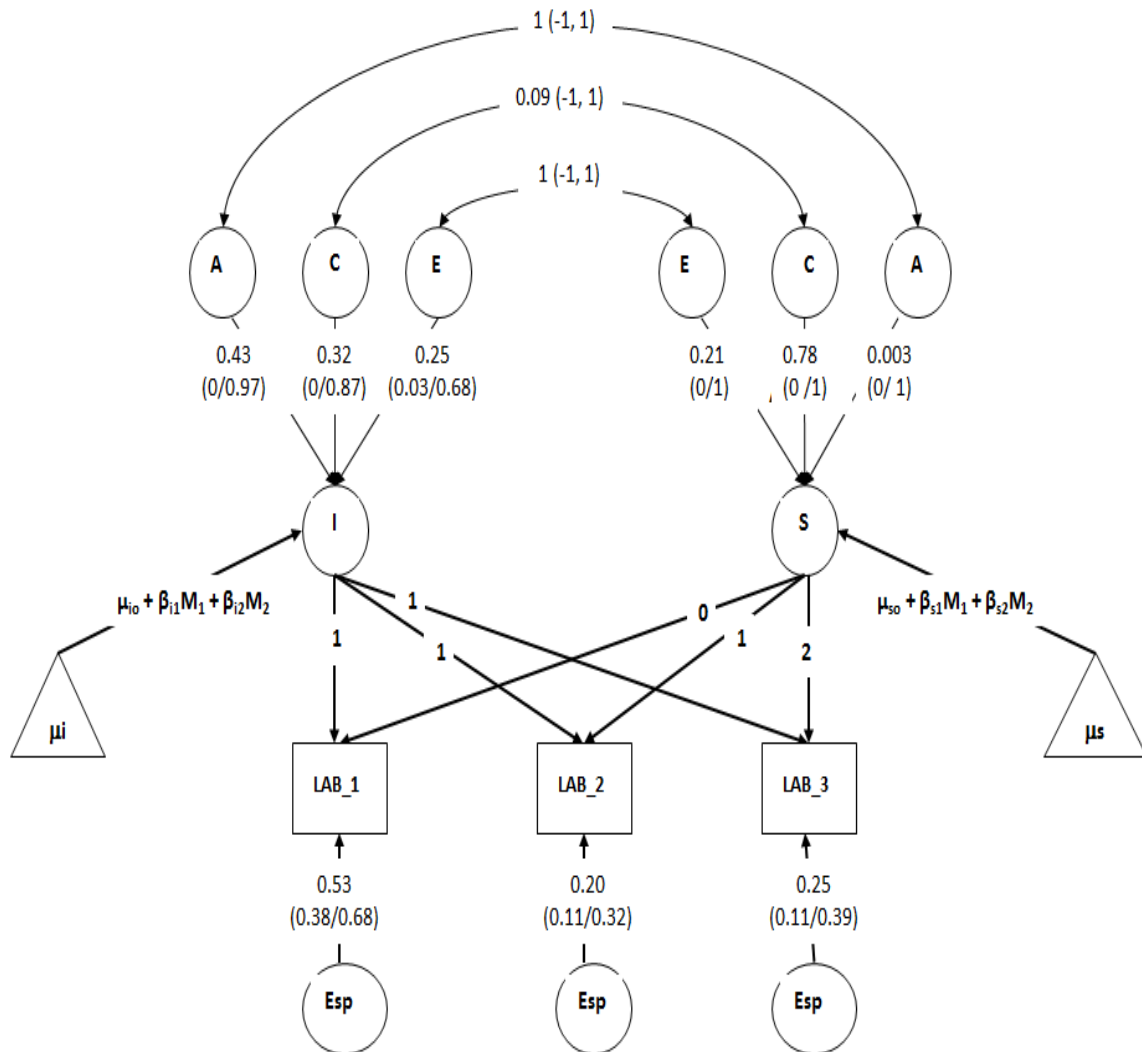
6.4.2.3.1 Aetiological components of growth curve factors

Additive genetic effects contributed substantially to the variance of the intercept (comprising the largest aetiological component, followed by shared environmental effects) (see Figure 6.2). In contrast, genetic influences were negligible for the slope, with over three-quarters of the variance accounted for shared environmental factors. However, CIs were wide and overlapped with zero, indicative of non-significance, which is likely attributable to insufficient sample size. The non-shared environmental and genetic correlations were estimated at unity, suggesting substantially overlapping aetiological factors influence both GCM factors (although CIs were wide and non-significant). The phenotypic correlation between the intercept and slope was modest and again non-significant (0.22 (-0.33/ 1.00)).

6.4.2.3.2 Aetiological components of the individual cortisol measures

The aetiological components of the individual lab measures can also be derived from this model (see Table 6.3). The familial components (A and C) were not significant for the individual cortisol measures, straddling zero. However, non-shared environmental influences (E), accounted for approximately 12% of the variation for the first sample, and a quarter of the variance of the last two samples. A significant proportion of the variance for individual cortisol samples is attributed to residual error (including measurement error), which was substantially high for the first laboratory cortisol measure (53%).

Figure 6.2 Genetic GCM for cortisol samples collected during cognitive-EEG testing and moderating effects of sampling conditions (age) and oppositional behavioural ratings incorporated as moderators in Intercept and Slope factor means



Note: LAB_1: baseline; LAB_2 sampled at end of cognitive tasks; LAB_3 sampled at end of assessments; Abbreviations- I: Intercept; S: Slope; Esp: Residual measurement error; μ_i : Intercept mean; μ_s : Slope mean; $\mu_{i0} + \beta_{i1}M_1 + \beta_{i2}M_2$, where μ_{i0} is the overall mean of the Intercept factor and β_{i1} and β_{i2} are the moderator 1 and 2 dependent means; $\mu_{s0} + \beta_{s1}M_1 + \beta_{s2}M_2$, where μ_{s0} is the overall mean of the Slope factor and β_{s1} and β_{s2} are the moderator 1 and 2 dependent means; Model presented for one twin only for ease of presentation;

Table 6.3 Aetiological components for individual cortisol sampled during cognitive-EEG session

	A	C	E	Esp
Lab_1	0.21 (0, 0.54)	0.15 (0, 0.47)	0.12 (0.01, 0.33)	0.53 (0.38, 0.68)
Lab_2	0.31 (0, 0.71)	0.23 (0, 0.63)	0.26 (0.11, 0.51)	0.20 (0.11, 0.32)
Lab_3	0.25 (0, 0.66)	0.24 (0, 0.59)	0.27 (0.10, 0.58)	0.25 (0.11, 0.39)

Note: A refers to additive genetic effects; C refers to shared environmental effects; E refers to non-shared environmental effects; Esp refers to residual measurement error; Lab_1 was a pre-task (baseline) measure; Lab_2 was obtained at end of cognitive testing; Lab_3 was obtained at end of the entire assessment;

6.4.2.4 Multivariate genetic model of ADHD affection status, oppositional behaviours and slope factor scores

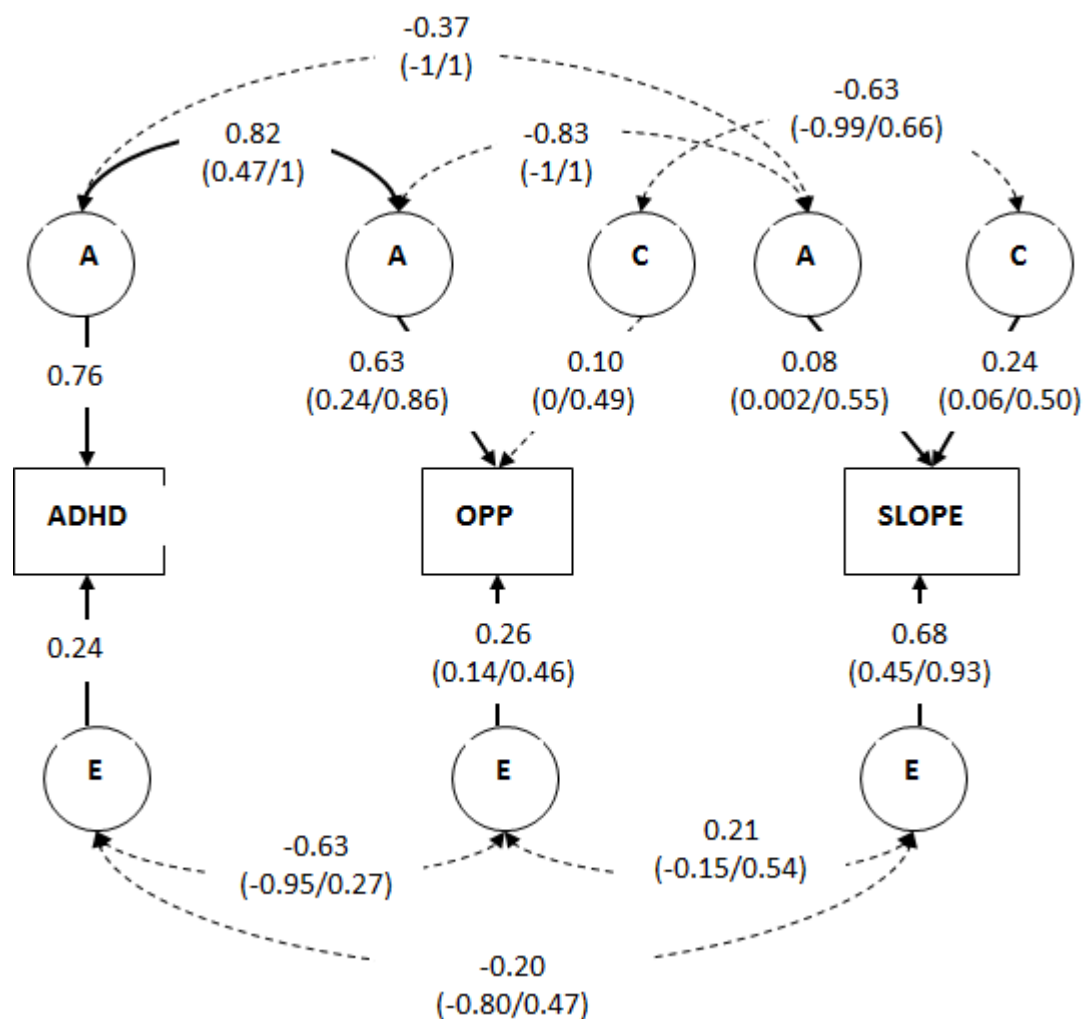
Based on our previous findings that oppositional behaviours moderated the main effect of ADHD affection status on the slope mean, we fitted a correlated factors solution model to ADHD affection status, oppositional behaviours and the derived slope factor scores to directly investigate the aetiological relationship between these three phenotypes (see Figure 6.3).

Oppositional behaviours showed strong genetic influences (63%), with the majority of the remaining variance accounted for by non-shared environmental effects. In line with our previous findings we found a small genetic component to the slope scores. However, in contrast with our previous findings we found that the majority of the variance in the slope was attributable to non-shared environmental factors, as opposed to shared environmental factors.

A substantial phenotypic (0.41 (0.24/0.57)) and strong genetic (0.82 (0.47/1.00)) correlation was observed between ADHD affection status and oppositional behaviours, in line with the previous literature.

The phenotypic correlation between the slope and ADHD affection status just missed significance (-0.17 (-0.35/0.02)), but just reached significance between the slope and oppositional behaviours (-0.19 (-0.37/-0.01)).

Figure 6.3 Correlated factors solution of the full Cholesky Decomposition across ADHD affection status, oppositional behaviours and slope factor scores

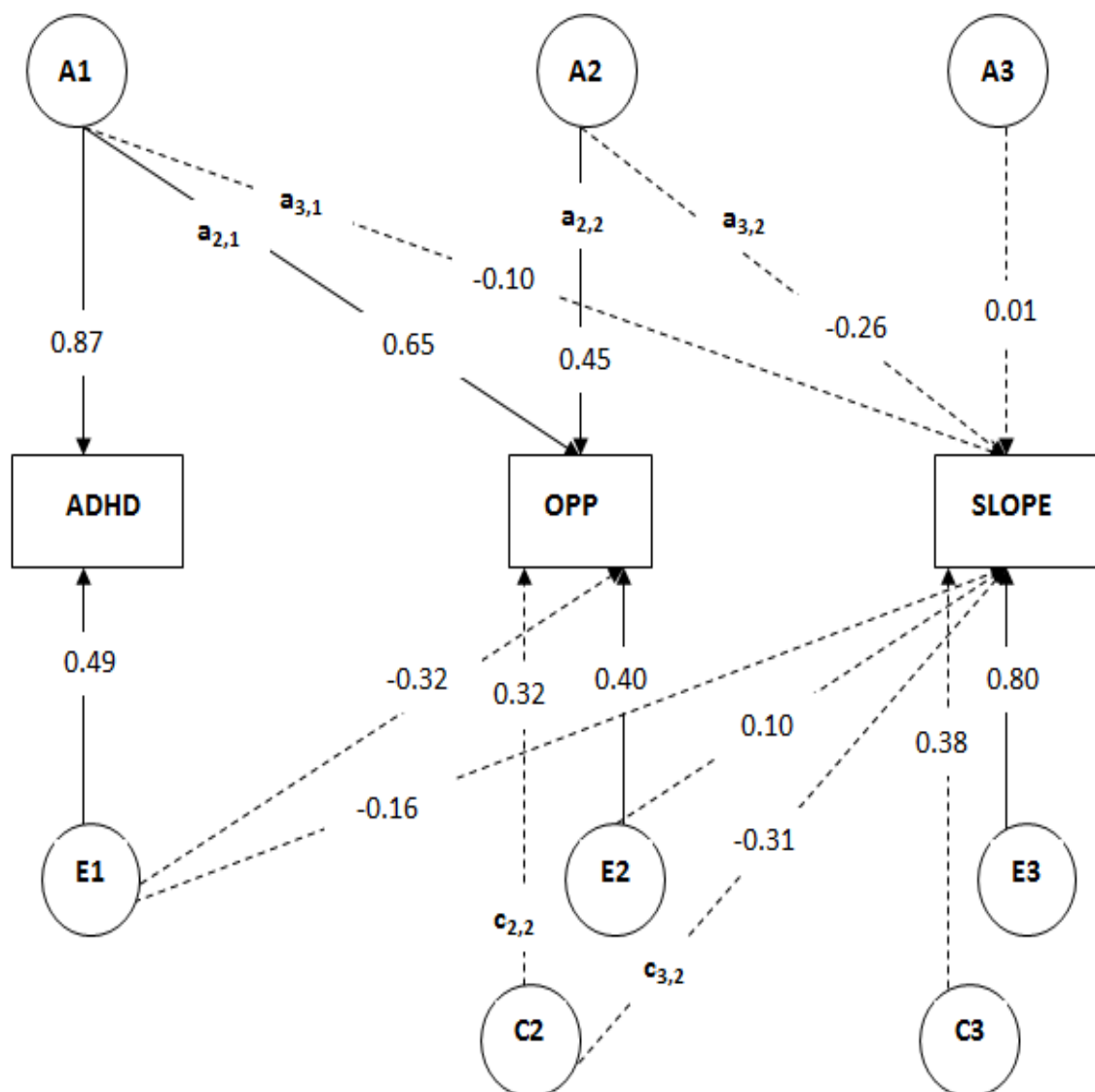


Note: Abbreviations: ADHD: ADHD affection status; OPP: oppositional behaviours; Significant parameters in solid lines; Non-significant parameters ($p = 0.05$) shown in dotted lines; Only shown for one twin for ease of presentation;

In terms of aetiological correlations between behavioural phenotypes and the slope factor, all these parameters were non-significant and it is likely that we have insufficient power to detect significance. The genetic correlations between ADHD affection status and the slope were negative, indicating that overlapping genes contribute to increased behavioural scores and a steeper slope. The same relationship was observed between oppositional behaviours and the slope. The genetic correlation between the slope and oppositional behaviours was more than double that observed between the slope and ADHD affection status, suggesting a higher proportion of overlapping genes. However, as mentioned it is important to note that CIs were wide, and non-significant.

It is likely that our non-significant aetiological correlations were a result of limited power. Using a Cholesky Decomposition (see Figure 6.4), we dropped A and C pathways from oppositional behaviours to the slope to identify if there was a significant familial component of covariance. The fit of models where individual Cholesky pathways were dropped were compared to the full model, and assessed by χ^2 difference test. Dropping all additive genetic pathways between oppositional behaviours and the slope (path $a_{2,2} * a_{3,2}$; and path $a_{2,1} * a_{3,1}$ (see Figure 6.4)), did not present a significant drop in fit compared to the full model. Dropping all C pathways between oppositional behaviours and the slope (path $c_{2,2} * c_{3,2}$), did not present a significant drop in fit compared to the full model. However, the model where all these parameters were dropped simultaneously did provide a significant worsening in fit ($\chi^2 = 7.62$, $df = 2$, $p = 0.05$), suggesting that there is a familial correlation.

Figure 6.4 Cholesky Decomposition of ADHD affection status, oppositional behaviours and slope growth curve factor scores



Note: All path estimates are standardised; Abbreviation: ADHD: ADHD affection status; OPP: oppositional behaviours; A1 and E1 relate to genetic and individual-specific environmental influences on all three phenotypes; A2, C2, and E2 relate to genetic and environmental influences that oppositional behaviours share with the slope; A3, C3 and E3 relate to genetic and environmental influences that are unique to the slope; Significant parameters in solid lines; Non-significant parameters ($p = 0.05$) shown in dotted lines; Only shown for one twin for ease of presentation;

6.4.2.5 Aetiological association between oppositional behaviours and the slope factor scores, independent of ADHD affection status

The interest in the common aetiological covariation of oppositional behaviours and the slope, independent of ADHD affection status was reflected in the order in which the variables were entered in the Cholesky Decomposition (see Figure 6.4). The first set of latent factors (A1, E1) represents genetic and environmental influences on ADHD affection status, as well as genetic and environmental influences shared between ADHD affection status, oppositional behaviours and the slope. The second set of latent factors (A2, C2, and E2) represents genetic and environmental influences on oppositionality and on its covariation with the slope, independent of aetiological effects shared with ADHD affection status. The final set of latent factors (A3, C3, and E3) represents genetic and environmental influences specific to the slope independent of both ADHD affections status and oppositional behaviours.

The product of the pathways from A2 to oppositionality and the slope indicates the genetic covariance between these two traits that is independent of ADHD affection status. The product of the pathways from A1 to oppositionality and the slope is the genetic covariance between these two phenotypes that are due to shared effects with ADHD affection status. The sum of these two estimates provides an estimate for the total genetic covariance between oppositional behaviours and the slope. To estimate how much of the genetic covariance between oppositional behaviours and the slope is independent of ADHD affection status, the genetic covariance between these two traits independent of ADHD affection status is divided by the total genetic covariance.

However, the mixture of negative and positive pathways prohibits this calculation. Accordingly, we calculated the covariance pathways that account for the covariance between oppositional behaviours and the slope that are shared with ADHD affection status, and those that are independent of ADHD affections status to see if they significantly differed. Therefore the genetic covariance between oppositional behaviours and the slope that is shared with ADHD affection status is $0.65 \times -0.10 = -0.07$ ($-0.25/0.10$), and that which is independent of ADHD affection status is $0.45 \times -0.26 = -0.12$ ($-0.44/0.17$).

The same can be done for non-shared environment: the individual-specific environmental covariance between oppositional behaviours and the slope that is shared with ADHD affection status is $-0.32 \times -0.16 = 0.05$ ($-0.14/0.25$), and that which is independent of ADHD affection status is $0.40 \times 0.10 = 0.04$ ($-0.19/0.26$).

The shared environmental (C) covariance between oppositional behaviours and the slope is only independent of ADHD affections status, and is $0.32 \times -0.31 = -0.10$ ($-0.36/0.0002$). All covariance pathways were non-significant. However, this covariance just missed significance, and it is likely that this covariance between the slope and oppositional behaviours would be significant in a larger sample. As there is no shared environmental factor component in ADHD affection status, we can speculate that the aetiological covariance between the slope and oppositional behaviours, if driven predominantly by shared environmental factors, will largely be independent of ADHD affection status.

6.5 Discussion

This is the first study to our knowledge to explore the aetiological overlap of the previously observed associations between atypical HPA axis activity and ADHD, and frequently co-occurring behaviours, and to employ growth curve modelling of cortisol sampled in ADHD-related populations. Six key findings emerged from our analysis.

Firstly, we did not find the expected group differences across the entire trajectory of cortisol concentrations over laboratory testing. The only significant group mean differences was found for measures excluding the first and incorporating the last sample, with the control group showing relatively stable cortisol levels between the two last sampling points, and the ADHD group showing a drop in cortisol concentration. In terms of diurnal variation, the ADHD group displayed an atypical change (drop) in cortisol levels from awakening to 30-minutes post-awakening, resulting in a negative cortisol awakening response, although this group difference did not retain significance after controlling for age and season of sampling (that showed group differences).

Secondly, we found that conventional indices of HPA axis activity (such as area-under the curve-based measures) were not significantly associated with ADHD affection status, and did not show significant genetic influences (as suggested by the low MZ correlations for cortisol measures).

Our third main finding, obtained using growth curve modelling, was that cortisol concentration levels during laboratory testing declined at a faster rate for the ADHD group, with a significantly steeper slope, compared to controls. In contrast, the intercept was similar across groups. Taken together, these findings suggest significant group differences relate to dynamic (degree of change) indicators of HPA functioning during cognitive testing rather than baseline levels. Increasingly, research suggests that reactivity measures, rather than single-point and absolute difference measures, are more ideally suited to capturing productivity (Balodis, Wynne-Edwards, & Olmstead, 2010), in line with our null findings using conventional measures of HPA axis activity. Our data suggests that GCM represents an alternatively effective means of examining cortisol activity.

Our fourth main finding was that when oppositional behaviours were simultaneously modelled as a moderator, we found a similar main effect of oppositional behaviours (as previously found for ADHD affection status) and that the main effect of ADHD affection status disappeared. There was also no interactive effect with oppositional behaviours and ADHD affection status. Therefore, our findings suggest that the association between ADHD affection status and a steeper decline is driven by oppositional behaviours. This is the first study to examine the moderating effects of oppositional behaviours on the association of ADHD with atypical HPA functioning, as captured by GCM, but mirrors other studies that suggest components of HPA activity associated with ADHD are actually explained by frequently co-occurring oppositional symptoms (Freitag et al., 2009; Snoek et al., 2004). However, a previous study concluded that hyperactivity-impulsivity and oppositional behaviours are phenotypically indistinguishable and largely index the same aetiological liability (Wood et al., 2009), raising the possibility that our findings may be reflective of an association with hyperactivity-impulsivity. We were unable to distinguish between ADHD behavioural dimensions in our study as children were selected for consistently high/low parental ratings across both symptom domains, yet the evidence from

other studies suggests that blunted cortisol activity in ADHD may indeed have a stronger relationship to hyperactivity-impulsivity, as opposed to inattentive symptoms (see section 6.2).

In contrast, anxiety-shy symptoms did not impact either GCM parameter, or moderate associations between the slope and ADHD affection status. The lack of a moderating effect of anxiety-shy symptoms on the association between ADHD affection status and cortisol reactivity is in line with some studies (Freitag et al., 2009; Pesonen et al., 2011), but in contrast with others (Hastings et al., 2009). However, it is important to note that in the only study to find an effect of a comorbid anxiety disorder in children with ADHD, this was only limited to those children that did not also have a comorbid disruptive behaviour disorder (Hastings et al., 2009). Thus, children with ADHD and comorbid anxiety disorder and ODD did not show increased cortisol reactivity, compared to children with comorbid ADHD and anxiety disorder alone. The other two studies were not able to group children according to the presence of both anxiety and disruptive behaviour disorders, as sample size was smaller.

Our sixth main finding was that the negative relationship between oppositional behaviours and the slope scores is primarily driven by familial factors. Moreover, we speculate that the familial covariance between oppositional behaviours and the slope was largely independent of shared effects with ADHD affection status. The familial nature is an important finding, and may contribute to our increasing understanding of neurobiological pathways underlying ADHD and co-occurring behaviours. We were unable to tease apart the familial component, potentially due to the small sample size, and as this is the first study to test the aetiological overlap of cortisol indices and ADHD/oppositional behaviours, replication with larger samples is needed before firm conclusions can be gleaned.

Despite the limited sample size, one of the main strengths of this study was that the sample was limited to include males only and to a restricted age range in order to reduce variability and maximize sample homogeneity. In addition, our sample was not based on clinical cases and thereby avoids biases associated with referral bias, and confounding effects of stimulant medication. It is important to emphasise that cognitive testing was part of a larger battery of assessment, and was not intended to provoke major stress. Yet, across both groups the first sample yielded the highest cortisol concentrations, suggesting potentially anticipatory stress

and HPA activation prior to entering the EEG lab. It is possible that the pattern of cortisol activity captured is reflecting only part of the stress-reactivity curve: subsequent recovery from the anticipation of entering the laboratory. Increased baseline levels may have subsequently obscured group differences in cortisol sampled during testing. In line with this, the only significant group mean differences were found for measures excluding the first and incorporating the last sample. Repeated sampling over a longer sampling time would allow a more accurate baseline assessment and the examination of reactivity and recovery.

In conclusion, this is the first study to apply the twin design to the examination of cortisol indices and ADHD and oppositional behaviours. Using GCM we found evidence for an association between the dynamic of cortisol productivity and ADHD affection status, although this was predominantly accounted for by oppositional behaviours. Moreover, we identified a familial component underlying oppositional behaviours and slope factor scores. An important direction for future research is to confirm a familial overlap and to tease apart the nature of the familial correlation, and the specificity of atypical cortisol output with externalising symptoms. Increased awareness of the pathophysiological processes involved in ADHD, may additionally contribute to facilitating improvements in treatment and management of ADHD (Corominas et al., 2012).

CHAPTER 7 GENERAL DISCUSSION

7.1 Abstract

The aim of this thesis was to utilise a multidisciplinary approach, combining behavioural ratings, genotyping data, cognitive-experimental and physiological measures, with both quantitative and molecular genetic analyses, to investigate underlying pathways from genes to behaviours related to attention-deficit/hyperactivity disorder (ADHD). Frequently co-occurring symptoms (oppositional behaviours, autistic-like traits (ALTs), and anxiety-shy symptoms) were also investigated to elucidate shared versus unique pathways, and moderators on potentially underlying processes. This chapter first summarises the key findings from the studies included in this thesis, followed by a discussion of the wider implications of our findings. Next, general limitations and strengths of the studies included in this thesis are outlined. This chapter will then conclude with an outline of future directions for ADHD research.

7.2 Summary of major findings

In chapter 2 the role of socio-demographic factors as contributory factors in parents' tendency to exaggerate behavioural differences when rating twin pairs according to ADHD symptoms was examined. This investigation indicated that within same-sex twin pairs, gender did not moderate contrast effects, suggesting that regardless of whether twin pairs are female or male they are similarly contrasted by parents. However, within opposite-sex pairs a gender effect was observed, with a significant effect from females-to-males and a non-significant effect from males-to-females, suggesting that within opposite-sex pairs, males are evaluated in relation to their female co-twin, but parents rate females independently of their male co-twins' behaviour.

A second key novel finding was that rater contrast effects were more likely to be present in parental ADHD ratings where family size was small. This effect was further moderated by gender composition: present in only female same-sex twin pair. Yet further research is needed to confirm that this is not an artefact of insufficient power to detect the same effect in twin pairs of other gender composition (see section 2.5 for more details)).

In line with previous findings, SES did not contribute to rater contrast effects (Saudino et al., 2004). We further replicated previous findings that longer and more detailed rating scales are less susceptible to rater contrast effects (Hay et al., 2007; Kuntsi, Rijdsdijk et al., 2005; Polderman et al., 2007), compared to shorter and more general ratings scales (Price et al., 2005; Price et al., 2001).

In chapter 3 using a population-based twin sample, we investigated whether two familial cognitive impairment factors previously identified in ADHD-combined (ADHD-C) subtype showed distinct genetic relationships to the two ADHD behavioural symptom domains of inattention and hyperactivity-impulsivity. We found that the association between ADHD and reaction time variability (RTV) largely reflect overlapping genetic effects between RTV and inattention ($r_G = 0.64$), with a less strong genetic overlap observed with hyperactivity-impulsivity ($r_G = 0.31$). Further analysis indicated that the majority (55%) of the genetic covariance between RTV and inattention was independent of genetic influences on hyperactivity-impulsivity. In contrast, genetic covariation between commission errors (CE) and both ADHD symptom domains showed less differentiation and were overall lower ($r_G = 0.11$ and $r_G = 0.17$ for inattention and hyperactivity-impulsivity, respectively). However, it is important to note that these correlations were non-significant. Further twin studies are required to clarify whether the low genetic correlations between CE and the ADHD symptom domains would emerge as significant in larger samples.

We extended the findings from chapter 3 by using a quantitative trait loci (QTL) study of the same variables in the same population-based twin sample (chapter 4). A similar pattern of genetic association findings emerged: nominal overlapping associations for CE and both ADHD behavioural dimensions with SLC6A2; and nominal overlapping associations for inattention and RTV with 5HT2A. These overlapping associations were then tested to distinguish between pleiotropy and mediating genetic effects. Further analyses suggested that the multiple nominal associations for SLC6A2 reflected pleiotropic genetic effects, whereas RTV mediated a substantial proportion of the effect of 5HT2A on inattention. However, none of the associations that emerged in the molecular genetic analysis remained significant after correcting for the number of markers tested, and we were unable to replicate associations with specific markers in an independent clinical sample.

In chapter 5, using the same population-based twin sample, we aimed to clarify the link between ADHD behaviours and autistic-like traits (ALTs). Non-social ALTs only showed a significant phenotypic correlation with hyperactivity-impulsivity, whereas social ALTs showed stronger correlations, equally with inattention and hyperactivity-impulsivity. A similar pattern emerged when examining the extent of overlapping genetic influences: social ALTs had substantial shared genetic effects with both ADHD behavioural dimensions, whereas non-social ALTs shared a lower degree of overlapping genetic influences, limited to hyperactivity-impulsivity. Our findings also suggested a moderate phenotypic correlation between social ALTs and RTV, in line with previous findings that suggest that RTV is not specific to ADHD. Furthermore, there were moderate overlapping genetic influences between RTV and social ALTs, but the majority of the genetic effects shared between social ALTs and inattention were independent of genetic effects shared with RTV.

In chapter 6 we examined a number of indicators of HPA axis activity, indexed by salivary cortisol, in a sample of children selected for consistently high versus low ADHD ratings. Our findings suggested that growth curve modelling was an effective means of capturing group differences, specifically in relation to the dynamics of HPA activity, with cortisol levels dropping significantly faster (slope mean) in the ADHD group. However, further analyses suggested that oppositional behaviours moderated the main effect of ADHD affection status on the slope mean, and largely drove the previously observed main effect of ADHD affection status. In line with this, although similar phenotypic correlations were obtained for the slope factor scores with both ADHD affection status and oppositional behaviours, it was only the latter association that reached significance. Model fitting suggested a familial component of covariance with individual slope scores and oppositional behaviours, although we had insufficient power to disentangle this familial correlation.

7.3 Wider implications for ADHD research

7.3.1 Increased knowledge of factors contributing to the inaccuracy of parental ADHD ratings

Only one other study has examined the role of parental socio-demographic factors, by means of correlations between parental difference scores in twin members' ADHD symptom ratings and parental socio-demographic factors, in a small sample of non-twin sib-pairs. In addition,

only one study has examined the role of gender on contrast effects within opposite-sex twin pairs (Rietveld, Hudziak et al., 2003). Therefore our study in chapter 2 was an important step in addressing the gaps in the literature.

In chapter 2 we investigated several socio-demographic factors, using multiple ratings across a number of time points in a large population-based twin sample. The study also employed a novel twin model to explicitly test for factors moderating contrast effects in parental ADHD ratings, by partitioning the total contrast effects observed into independent and moderator-dependent components. Our findings suggested that gender moderated rater contrast effects within opposite-sex twin pairs, but the direction of effect was in the opposite direction to the previous finding in the literature (Rietveld, Hudziak et al., 2003). This is discussed in more detail in section 2.5, but underlines the importance of attempting to replicate novel findings in independent samples.

Our finding that family size contributed to rater contrast effects provides further evidence that contrast effects likely reflect perceptual rather than actual behavioural differences (see section 1.3.7.2). Our findings also converge with other studies to empirically highlight the biases associated with rating scales and parental ratings, and underline the use of longer more detailed rating scales that are less prone to contrast effects, obtaining ratings for multiple informants, and directing attention at objective ADHD-related measures.

7.3.2 Validation of the dimensional approach to ADHD

A number of our findings derived from analysis of population-based samples converge with previous observations based on clinical samples, providing additional support to validate a dimensional concept of ADHD, where ADHD is viewed as the extreme of a quantitative dimension (see section 1.3.2). These include, but are not limited to, the familial separation of RTV and CE, and the strong genetic overlap between MRT and RTV (Kuntsi et al., 2010). This has implications for our understanding of the nature of ADHD by demonstrating the quantitative nature of ADHD at both the aetiological and phenotypic (cognitive and symptom) level. Furthermore, in chapter 4 we found significant associations with previously implicated ADHD risk markers, adopting a QTL approach in the same population-based sample. Although

none of these associations withstood correction for multiple testing and we were unable to find overlapping findings in an independent clinical sample, the associations yielded from the analysis of a population sample, suggest that previous association findings derived from clinical ADHD may extend to quantitative ADHD-related traits.

The generalisability of quantitative genetic findings across clinical and population-based samples, and significant associations with putative ADHD risk markers in a population-based sample, are in line with the dimensional view of ADHD behaviours, and underline that population-based samples are a complementary alternative to build causal models and investigate underlying processed underlying ADHD, rather than one approach (clinical versus population-based) having the upper hand.

7.3.3 Supporting the separation of ADHD symptom subscales

The majority of clinical samples consist of individuals with ADHD-C subtype, and therefore much of what we know about ADHD pertains to this subtype. Although there have been a few comparative studies of neuropsychological and molecular genetic correlates according to ADHD subtypes, the majority have had too small numbers of people with predominantly hyperactivity-impulsivity (ADHD-PHI) subtype. Therefore an important gap in the literature that requires addressing is phenotypic heterogeneity in relation to cognitive processes in ADHD, and the identification of subtype- specific and common genetic susceptibility loci.

An alternative approach to subtype comparisons is to study the underlying behavioural dimensions of inattention and hyperactivity-impulsivity, using the full range of symptom scores in general population samples (Chen et al., 2008). The findings from chapter 3 to 5 support the separation of the ADHD symptom subscales of inattention and hyperactivity-impulsivity. For example, the majority of the genetic covariance between RTV and inattention was independent of genetic influences on hyperactivity-impulsivity (chapter 3), emphasising that largely separate genetic effects account for the covariance between inattention and RTV on the one hand, and between inattention and hyperactivity-impulsivity on the other. In chapter 4, nominal molecular genetic associations were shared across both inattention and hyperactivity-impulsivity, and specific to behavioural domains.

Taken together, these findings highlight that inattention and hyperactivity-impulsivity have partially dissociable underlying genetic influences and correlates, and emphasise that theoretical models of ADHD should focus increased attention at multiple pathways.

7.3.4 Contribution to cognitive theoretical models of ADHD

The findings from chapter 3 and 4 converge with previous studies using clinical ADHD, underlining the importance of both shared and unique aetiological pathways on the two symptom domains of ADHD. Moreover our quantitative genetic analyses suggest that RTV underlies inattention, which is in line with hypotheses that cognitive factors underlie ADHD symptoms (see section 1.5). This pathway may explain the developmental persistence of inattention (see section 1.2.2.3), in line with the neurodevelopmental model of ADHD (see section 1.5.3) that views RTV as a persistent impairment in ADHD (Halperin & Schulz, 2006; Halperin et al., 2008).

A number of theoretical models implicate deficits in arousal dysfunction as contributing to the observed cognitive impairments associated with ADHD (see section 1.5.2). Salivary cortisol can be considered as a physiological indicator of arousal, and our findings from chapter 6 underline the importance of teasing out whether underlying processes are due specifically to ADHD symptoms or (moderated) by commonly co-occurring behaviours.

7.3.5 Clarifying the link between ADHD and ASD

A limitation of existing twin studies investigating the genetic overlap between these two phenotypes is that they fail to take into account the genetic heterogeneity observed within both ADHD and ALT symptom subscales. In chapter 5 we addressed this limitation, separating ADHD and ALT symptom subscale ratings. Our findings underlined the need to separate symptom subscales for other phenotypes, and also suggest that the aetiology of disorders do not necessarily follow diagnostic boundaries.

The search for shared endophenotypes across ADHD and autism spectrum disorders (ASDs) has been hypothesised to help facilitate the search for pleiotropic genes and shared underlying risk

pathways (Rommelse et al., 2011). Our findings suggested that RTV may represent a common cognitive process underlying both disorders.

7.3.5 RTV and CE as ADHD endophenotypes

In section 1.6 we evaluated RTV and CE as ADHD endophenotypes according to existing criteria. Overall, the reviewed evidence was stronger for RTV across the entire criteria, although we noted that the two-factor model derived from analysis on a clinical sib-pair sample suggests that RTV cannot fully account for ADHD, but that accuracy-related factors, such as CE, are also involved. Our findings provide further evidence underlining both RTV and CE as underlying processes involved in ADHD. In line with existing literature our findings suggested that relative to CE, RTV is a more promising endophenotype for ADHD, although RTV may also be an endophenotype for subscales of other traits (such as social ALTs). Furthermore, our findings suggest that RTV is likely an intermediate phenotype (rather than a risk indicator) of inattention.

7.3.6 The identification of potential new endophenotypes

In section 1.6 we outlined the criteria used to assess the validity of endophenotypes. Despite increasing research examining HPA axis functioning in ADHD/ODD, no study has yet investigated the aetiological overlap of indices of HPA activity and ADHD/ODD. This is an important gap in the literature; if overlapping genetic effects can be identified, and HPA axis activity clarified as mediating genetic effects (rather than representing pleiotropic genetic effects), this may represent a target for treatment strategies.

Our findings suggested a familial component of covariance between the rate of change in cortisol levels across a laboratory session and oppositional behaviours. This finding needs to be replicated in a larger sample. Moreover, a genetically sensitive design can be employed to disentangle the familial correlation into shared genetic or environmental effects. .

7.4 Strengths and limitations

7.4.1 Limitations of the twin method

The analyses within this thesis are subject to the standard limitations of the twin method (reviewed in section 1.3.1.6), including, but not limited to, the equal environments assumption, non-assortative mating, and the generalisability of findings from twins.

In a twin study on hyperactivity symptom scores, MZ twins were found to share a greater degree of environmental similarity than DZ twin pairs. However, subsequent analysis identified that the environmental sharing score was not correlated with MZ twin similarity for hyperactivity rating scores (Thapar, Hervas, & McGuffin, 1995), underlining that deviations of the equal environments assumption are unlikely to contribute to major biases in parameter estimates.

In a study including parent data, a spousal correlation of 0.11 was estimated for ADHD scores, suggestive of the presence of assortative mating (Boomsma et al., 2010). However, in a study on major psychiatric diagnoses while there was evidence for assortative mating effects both within and across major psychiatric disorders, the effect was largely negligible (Maes et al., 1998). Moreover, results from population-based studies are less likely to be affected by non-random assortment.

As previously mentioned (see section 1.3.1.6), compared to singletons, twins tend to show delays in language attainment and cognitive ability, although this group difference is absent by middle childhood (Plomin et al., 2008). The cognitive assessments of the samples included in the remainder of this thesis (chapter 3 to 5) were conducted no earlier than age 6. Therefore, it is unlikely that any of our assessments were influenced by cognitive developmental delays. However, mixed findings have been reported regarding the effect of twinning on ADHD risk. For example, one study found differences in correlations for ADHD symptoms between twins and non-twin sibling pairs, with lower correlations for non-twin siblings (for both males and females) (Levy et al., 1997), implicating a twin-specific effect. More recently, using the same approach, no twin-specific effect was found for ADHD ratings (Ehringer, Rhee, Young, Corley, & Hewitt, 2006).

Taken together, it is likely that any bias due to the above limitations to parameter estimates is minimal. Accordingly, we conclude that the limitations of the twin design do not detract from its advantages.

7.4.2 Sample characteristics and measurement issues

For the most part, the studies included in this thesis were based on population-based twin samples which avoid biases inherent with clinical samples, such as a greater disproportion of males (see section 1.2.2.2) and the potential influences of genetic risk factors with other comorbid disorders. As already highlighted (section 7.3.3), the use of population samples enabled an investigation of the two ADHD symptom dimensions, and the two ALT symptom subscales, separately.

Although caution is required when extrapolating findings from general population-based to clinical samples, there is evidence to suggest that ADHD genetic liability is continuously distributed and that ADHD can be viewed as lying at the tail end of a continuous dimension. Moreover, as already discussed (see section 7.3.2), we were able to replicate findings from a clinical sample in our investigation of a population-based sample, further supporting the quantitative nature of ADHD symptoms.

In our study on HPA axis activity (chapter 6), included twin pairs were selected on the basis of consistently high or low parental ADHD ratings across both inattentive and hyperactive-impulsive domains. The selected sample design contributes to increased power for studying relatively low prevalence disorders (Neale, Eaves, & Kendler, 1994). Despite the greater statistical power, our sample size was particularly small, and likely contributed to insufficient power to identify significant aetiological correlations. In addition, as twin pairs were selected for consistently high/low symptom scores across both inattention and hyperactivity-impulsivity, we did not investigate the overlap of HPA axis indicators with the specific behavioural dimensions of ADHD.

The samples used in all the other studies included in this thesis were relatively large. All ADHD ratings included in the studies in this thesis were based on DSM-IV ADHD ratings, which we

showed in chapter 1 are less susceptible to rater contrast effects, in line with previous research. Moreover, previous research has indicated that DSM-IV based interview and questionnaire-based measures largely index the same genetic liability (Derks et al., 2008). In addition, there is substantial evidence to suggest that ADHD symptoms can be viewed as continuously distributed traits (see section 1.3.2).

For the most part (chapters 3 to 5), composite behavioural measures were used based on summed parent and teacher ratings. However, inter-rater correlations were medium for ADHD ratings (around 0.40), but lower (around 0.20) for ALTs (chapter 5). Informant effects was not a focus of our studies, and the decision to combine scores across informants was done so as to capture a more stable, reliable and situational pervasive measure of problem behaviours.

In addition, it is important to note that the majority of assessments of twin samples were conducted during middle childhood and adolescence, and therefore cannot be extrapolated to ADHD in adulthood.

7.5 Future directions

As a number of the findings generated from the studies included in this thesis are novel (highlighted in section 7.3), further studies are needed in independent samples to confirm our findings.

In addition, further studies are needed to extend our findings. For example, additional socio-demographic variables can be investigated as factors contributing to parental contrast effects in ADHD ratings, such as parental occupation and age. In addition, future work should clarify whether the shared genetic effects between RTV and social ALTs (chapter 5) represent pleiotropy or mediation. In addition, future studies should clarify whether traits associated with ADHD show distinct aetiological and phenotypic covariation with inattention and hyperactivity-impulsivity; and clarify whether ADHD endophenotypes covary with the disorder, or lie of the causal risk pathway between aetiological factors.

Furthermore, the studies included in this thesis are cross-sectional, and longitudinal studies are needed to clarify whether the genetic correlations identified in chapters 3 and 5 are stable across the life-span or change across development.

In particular the findings from this thesis should be incorporated with structural and functional brain studies, as this will provide further knowledge about the neuropsychology of ADHD. In addition, extending these investigations into animal studies will enhance our understanding of the functional role of ADHD associated genetic susceptibility loci at the level of psychological processes.

7.6 Overall conclusion

The study of the genetic aetiology of ADHD with both quantitative and molecular genetic approaches, and the cognitive-psychological and physiological processes involved in ADHD, have often been studied in isolation from one another. It is only comparatively recently that investigations have studied these areas in conjunction with one another, and therefore there is an emerging body of research on the links between them. This thesis combines both quantitative and molecular genetic approaches with genotyping data, behavioural ratings, and cognitive-experimental and physiological measures, and highlights that multidisciplinary investigations are useful to identify the psychological and physiological processes underlying ADHD and their association with ADHD aetiological risk factors.

The main objective of this thesis was to identify pathways from genes to ADHD behaviours, separating both behavioural dimensions, and bridging the gap between molecular genetic findings and behavioural outcomes. Accordingly, the majority of the studies included in this thesis (chapters 3 to 6) investigated ADHD candidate endophenotypes. In particular, one key focus of my research was directed at utilising an endophenotype approach to clarify the processes that underlie the two main familial ADHD cognitive factors, RTV and CE, identified to date (Kuntsi et al., 2010). Our findings highlighted the importance of both shared and unique aetiological pathways to the two symptom domains of ADHD, indicated by separable genetic pathways related to these cognitive factors, specifically CE underlying both behavioural dimensions, and RTV underlying inattention. Furthermore, our findings showed further distinctions reflected in patterns of associations with co-occurring traits, in particular social

ALTs is equally associated with both ADHD behavioural symptoms, and RTV is not unique to ADHD, but shared with social ALTs.

These findings enhance our understanding of the aetiological and neurocognitive processes underlying ADHD and frequently co-occurring neurodevelopmental disorders, linking associations with overlapping genetic effects, but further describing their relationship to distinct behavioural outcomes. These findings further underline that current diagnostic boundaries do not map onto aetiological boundaries, and accordingly may give rise to different ways of conceptualising these disorders, particularly current approaches of diagnostic classification. Thus, the identification of shared versus unique processes underlying multiple behavioural outcomes, may also be used to stratify samples into more homogenous subgroups, even across diagnostic constructs, rather than based on symptom presentation.

Findings derived from molecular genetic analyses in this thesis show both the promise in potentially elucidating gene-cognitive-behavioural pathways (such as the potentially mediating pathway between 5HT2A to inattention, via RTV), but also the challenges, as the molecular genetic associations did not retain significance after correcting for multiple testing and findings did not replicate across independent samples.

Overall, although the QTL approach may be a complementary alternative to targeting clinical derived categories with putative ADHD risk markers, linking molecular genetic mechanisms to neurocognitive processes that underlie complex behavioural outcomes, the main appeal of endophenotypes is in elucidating underlying pathophysiological processes.

A further implication of our findings for molecular genetic strategies is that when parent ADHD ratings are utilised, greater efforts should be made to use a rating scale more resistant to rater contrast effects.

Although a number of cognitive models of ADHD have implicated deficits in arousal dysregulation (see section 1.5.3), our final set of empirical findings suggested atypical arousal as indexed by salivary cortisol, which previous studies have suggested is associated with ADHD,

is explained by oppositional behaviours. Research on HPA axis functioning in ADHD is comparatively small; yet our findings highlight the promise of this approach.

Taken together, our findings contribute to guiding the direction of the research agenda for future studies: clarifying the complex underlying pathways from genes to ADHD, and providing an important step in advancing our understanding of ADHD. Given the associated personal and social burden associated with ADHD, research on ADHD should continue to remain a high priority.

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APPENDIX A:**Table A.1 Genetic markers chosen for genotyping in population-based twin sample ^a**

Gene	Marker	Reference for previous associations with clinical ADHD
CDH13	rs6565113	Association with total symptom count (Lasky-Su, Neale, et al., 2008).
CDH13	rs11646411	Associated with adult ADHD (Lesch, et al., 2008).
CNTFR	rs7036351	Associated with both adult and childhood ADHD (Ribases et al., 2008).
DAT1	Intron 8 VNTR	Associated with clinical ADHD in meta-analysis (Gizer, et al., 2009).
DAT1	3'UTR VNTR	Associated with clinical ADHD in meta-analyses (Gizer, et al., 2009; Yang et al., 2007).
DRD4	Exon 3 VNTR	Associated with clinical ADHD in meta-analyses (Gizer, et al., 2009; Li, et al., 2006)
HTR1B	rs6296	Associated with clinical ADHD in meta-analysis (Gizer, et al., 2009).
MAOA	rs6323	Gene associated with ADHD (Domschke et al., 2005; Xu et al., 2007).
SLC6A2	rs3785143	Associated with clinical ADHD (Brookes et al., 2006; Kim et al., 2008; Xu et al., 2008).
SLC6A2	rs3785157	Associated with clinical ADHD (Bobb, et al., 2005; Xu, Knight, et al., 2005), but with opposing alleles.
SNAP-25	rs1051312	Association found when 5 independent studies pooled together (Kim et al., 2007).
SNAP-25	rs6077690	Association found when 5 independent studies pooled together (Kim, et al., 2007).
TPH2	rs1843809	Associated with clinical ADHD (Sheehan et al., 2005). + (Brookes et al., 2006), but with opposing allele
5HT2A	rs7322347	Associated with ADHD-C subtype in children (not adults) (Ribases, et al., 2009).
5HT2A	rs7984966	Associated with ADHD-C subtype in adults (not children) (Ribases, et al., 2009).

Note: Excluded markers are not shown; ^a for analysis in chapter 4, part A

APPENDIX B:

Table B.1 Maximum-likelihood correlations and 95% confidence intervals (constrained correlational model)

	Hyperactivity- Impulsivity ^b	Inattention ^b	ASD Social ^b	ASD Non-social ^b	RTV ^c	CE ^d
Twin correlations						
MZ/DZ	Twin 1					
	Twin 2					
Hyperactivity- impulsivity ^b	0.73 (0.66/0.90) <i>0.30 (0.21/0.39)</i>	<i>0.16 (0.08/0.24)</i>	<i>0.14 (0.06/0.22)</i>	<i>0.08 (-0.02/0.15)</i>	<i>0.05 (-0.03/0.12)</i>	<i>0.04 (-0.03/0.07)</i>
Inattention ^b	0.45 (0.38/0.51)	0.63 (0.54/0.67) <i>0.09 (0.02/0.15)</i>	<i>0.13 (0.04/0.21)</i>	<i>0.06 (-0.02/0.10)</i>	<i>0.03 (-0.05/0.10)</i>	<i>0.01 (-0.07/0.08)</i>
ASD Social ^b	0.31 (0.23/0.37)	0.31 (0.23/0.35)	0.72 (0.65/0.77) <i>0.30 (0.20/0.40)</i>	<i>0.11 (0.03 - 0.18)</i>	<i>0.03 (-0.05/0.10)</i>	<i>-0.01 (-0.08/0.07)</i>
ASD Non-social ^b	0.13 (0.05/0.19)	-0.01(-0.09/0.03)	0.15 (0.07/0.25)	0.66 (0.58/0.73) <i>0.29 (0.18/0.39)</i>	<i>0.01 (-0.07/0.06)</i>	<i>0.02 (-0.06/0.08)</i>
RTV ^c	0.14 (0.06/0.21)	0.19 (0.11/0.26)	0.16 (0.08/0.24)	-0.01 (-0.10/0.05)	0.43 (0.33/0.52) <i>0.23 (0.12/0.29)</i>	<i>0.05 (-0.02/0.13)</i>
CE ^d	0.06 (-0.02/0.14)	0.04 (-0.04/0.11)	0.01 (-0.07/0.09)	-0.05(-0.13/-0.01)	0.02 (-0.06/0.11)	0.28 (0.16/0.37) <i>0.13 (0.03/0.23)</i>

Note: Due to the lack of quantitative and qualitative sex differences, MZ and DZ correlations are not presented by sex. ^a Estimated using maximum likelihood estimation; ^b Sum of parent and teacher ratings; ^c Sum of unstandardised data scores across baseline condition of fast task and slow condition of the go/no-go task; ^d Sum of percentages of CE across slow and fast condition of the GNG task; Abbreviations: MZ: monozygotic DZ: dizygotic; ASD Social: social subscale of autistic-like traits; ASD Non-Social: non-social subscale of autistic-like traits; RTV; reaction time variability; CE: commission errors; **MZ data in bold typeface, DZ data in italic typeface**

APPENDIX B:**Table B.2 Aetiological and phenotypic correlations (standardised correlated factors solution genetic ACE model) for all variables**

	Hyperactivity- impulsivity^a	Inattention^a	ASD Social^a	ASD Non-Social^a	RTV^b	CE^c
Phenotypic correlations						
Inattention ^a	0.58 (0.54/0.62)	-	-	-	-	-
ASD Social ^a	0.30 (0.23/0.37)	0.32 (0.26/0.39)	-	-	-	-
ASD Non-Social ^a	0.11 (0.04/0.19)	-0.06 (-0.13/0.01)	0.17 (0.11 /0.24)	-	-	-
RTV ^b	0.17 (0.11/0.23)	0.25 (0.19/0.30)	0.18 (0.11/0.25)	-0.04 (-0.11/0.03)	-	-
CE ^c	0.09 (0.03/-0.15)	0.12 (0.06/0.18)	0.02 (-0.05/0.09)	-0.09 (-0.15/-0.02)	0.12 (0.07/0.18)	-
Broad-sense genetic influences						
Hyperactivity- impulsivity ^a	0.72 (0.66/0.77)	0.44 (75%)	0.32 *	0.14 *	0.14 (81%)	0.06 (70%)
Inattention ^a	0.73 (0.64/0.81)	0.51 (0.41/0.61)	0.31 (96%)	0.03 *	0.17 (68%)	0.04 (30%)
ASD Social ^a	0.44 (0.33/0.55)	0.52 (0.39/0.65)	0.71 (0.64/0.77)	0.16 (89%)	0.16 (87%)	0.04 (71%)
ASD Non-social ^a	0.20 (0.08/0.32)	0.05 (-0.11/0.21)	0.23 (0.11/0.35)	0.65 (0.56/0.72)	-0.01 (13%)	-0.04 (42%)
RTV ^b	0.28 (0.12/0.57)	0.40 (0.21/0.81)	0.32 (0.15/0.66)	-0.01 (-0.20/0.21)	0.34 (0.09/0.51)	-0.04 *
CE ^c	0.16 (-0.03/0.84)	0.11 (-0.18/0.71)	0.12(-0.35/0.37)	-0.10 (-0.68/0.15)	-0.16 (-0.94/0.43)	0.21 (0.01/0.36)
Common environmental influences (cognitive variables)						
Hyperactivity- impulsivity ^a	-	-	-	-	-	-

Inattention ^a	-	-	-	-	-	-
ASD Social ^a	-	-	-	-	-	-
ASD Non-social ^a	-	-	-	-	-	-
RTV ^b	-	-	-	-	0.09 (0/0.28)	0.07 *
CE ^c	-	-	-	-	1 (-1.00/1.00)	0.05 (0/0.22)
Individual-specific environmental influences						
Hyperactivity-impulsivity ^a	0.28 (0.23/0.35)	0.14 (25%)	-0.01 *	-0.03 *	0.03 (19%)	0.03 (31%)
Inattention ^a	0.39 (0.27/0.50)	0.49 (0.39/0.59)	0.01 (4%)	-0.08 *	0.08 (32%)	0.08 (70%)
ASD Social ^a	-0.03(-0.17/0.11)	0.03 (-0.12/0.18)	0.29 (0.24/0.36)	0.02 (11%)	0.02 (13%)	0.01 (29%)
ASD Non-social ^a	-0.08(-0.21/0.07)	-0.20 (-0.34/-0.05)	0.06 (-0.09/0.22)	0.35 (0.28/0.44)	-0.03 (87%)	-0.05 (58%)
RTV ^b	0.08 (-0.04/0.20)	0.15 (0.03/0.26)	0.06 (-0.07/0.19)	-0.07 (-0.20/0.06)	0.58 (0.49/0.68)	0.10 *
CE ^c	0.06 (-0.06/0.18)	0.14 (0.03/0.25)	0.04 (-0.09/0.16)	-0.10 (0.22/0.03)	0.15 (0.04/0.26)	0.74 (0.64/0.85)

Note: In the upper part of the table, the phenotypic correlations, as estimated by the full correlated factors model, are given. In the next quarter, the additive genetics (with 95% confidence intervals (CIs)) of each variable is given on the diagonal. The additive genetic correlations between the pairs of variables (with 95% CIs) are given below the diagonal. The contribution of additive genetic factors to the phenotypic correlation between variables is given above the diagonal, with the percentage of the phenotypic correlation that is due to additive genetic effects in brackets. The same information is presented for shared environmental and individual-specific environmental influences in the third and lower quarters of the table, respectively. Significant estimates in bold; * It was not possible to formally estimate these proportions, due to the presence of both positive and negative aetiological correlations between relevant variables; ^a Sum of parent and teacher ratings; ^b Sum of unstandardised data scores across baseline condition of the fast task and slow condition of the go/no-go (GNG task); ^c Sum of percentages of CE across slow and fast conditions of GNG task; Abbreviations: ASD Social: social subscale of autistic-like traits; ASD Non-social: non-social subscale of autistic-like traits; RTV; reaction time variability; CE: commission errors;

APPENDIX C:**SUPPLEMENTARY INFORMATION ON GROWTH MIXTURE MODELLING ON LONGITUDINAL TEDS ADHD DATA**

Latent Class Analyses (LCA) on longitudinal data can be used to identify subgroups in the sample that show different developmental trajectories. LCA was used to optimally select either concordant high or low ADHD twin pairs for our study in chapter 6. LCA were applied on three time points of data (i) hyperactivity-impulsivity; (ii) inattention and (iii) ADHD. The log-transformed total score of from a DSM-IV measure of ADHD as calculated on boys only (age eight, 12, 14), with medical cases excluded. The LCA were conducted on individual data, with the COMPLEX analysis option in Mplus to account for the non-independence of observations and with missing data managed through Full Information Maximum Likelihood.

The analyses typically involve fitting a series of models, starting with one and moving to multiple class models. The most parsimonious number of classes can be selected by means of a number of fit indices as well as usefulness (interpretation) of classes and previous findings in the literature. In this case, for the purpose of selection, for all three scales we opted for a three-class model where consistently high, low and middle class of individuals were clearly identified. The output takes the form of both a posterior probability belonging to each class and an assigned 'class or trajectory membership' based on the highest probability. Class membership proportions were as follows: Inattentive subscale: low class, 17.7%; middle class, 38.2%; high class, 44.1%; Hyperactive/Impulsive subscale: low class, 20.3%; middle class, 46.4%; high class, 33.3%.

We then selected twin pairs where both twins were from the consistently high class of combined hyperactivity-impulsivity and inattention symptom scores (concordant for ADHD); twin pairs who were both from the consistently low class of combined hyperactivity-impulsivity and inattention symptom scores (control pairs) and pairs where one twin was from the high class and the co-twin was from the low class (discordant pairs).